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16. ABSTRACT

The objective of this research was to assess the effects on freshwater algae of materials derived from coal storage piles. Coal leachates and distillates were prepared in the laboratory from low-sulfur Montana coal. Three types of algal bioassays were conducted:

1) A laboratory stream facility was constructed which supported periphyton communities of 50-80 species growing on artificial substrates. These communities generally showed stimulation of growth and some species composition changes in response to coal leachates. Coal distillates inhibited growth. Periphyton exposed to distillates accumulated aliphatic hydrocarbons.
2) Short-term laboratory bottle tests with test species of algae generally showed growth inhibition in response to leachates and distillates. When distillates were bubbled to remove volatile organic compounds, growth stimulation was observed.
3) Three in situ experiments in a small lake were conducted with coal distillates. Increases in algal biomass and bacterial populations in distillate-treated enclosures were observed in each of these tests.

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ALGAL BIOASSAYS WITH LEACHATES AND DISTILLATES FROM WESTERN COAL

by

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FOREWORD

Our nation's fresh waters are vital for all animal and plant life, yet our diverse uses of water -- for recreation, food, energy, transportation, and industry -- can physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as the aquatic ones. The Environmental Research Laboratory in Duluth, Minnesota, develops methods, conducts laboratory and field studies, and extrapolates research findings from both inhouse and extramural projects

-- to determine how physical and chemical pollution effects aquatic life;

-- to assess the effects of ecosystems on pollutants;

-- to predict effects of pollutants on the ecosystems through use of models; and

-- to measure the rate of uptake and bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man.

A comprehensive program was designed in 1974 because of the "Energy Crisis" of 1973 to study the adverse effects on the aquatic environment being created by new energy sources and technologies. Data being reported in this grant are a part of the comprehensive program and deal with the possible effects of coal storage on periphyton.

Laboratory periphyton communities were generally stimulated by coal leachates and inhibited by coal distillates. Whereas distillates and leachates were toxic to algae in bottle tests. In contrast, in situ experiments with natural phytoplankton and coal distillates indicated stimulation.

These findings will be integrated with other studies to aid elected officials in making environmentally sound decisions on future energy developments.

J. David Yount, Ph.D.
Acting Director
Environmental Research Laboratory-Duluth

ABSTRACT

The objective of this research was to assess the effects on the aquatic environment of materials derived from coal storage piles, and specifically, the effects of these materials on freshwater algae. Coal leachates and distillates were prepared in the laboratory from low-sulfur western coal from Montana. Leachates prepared from this coal had conductivities of 70-350 μmhos/cm and contained appreciable quantities of sodium and sulfate ions, organic phosphorus, and coal dust. The pH values for leachates varied between 7.0 and 7.7, and concentrations of metals and volatile organics were low. Coal distillates, prepared by boiling a coal/water mixture and collecting the condensate, had conductivities of 5-30 μmhos/cm, pH values of 4.5-5.5, and contained volatile organic compounds. Total organic carbon in distillates ranged from 5-34 mg/1.

Three types of bioassays were conducted to determine the effects of coal leachates and distillates on algal growth:

1) A laboratory stream facility was designed and constructed which supported periphyton communities of 50-80 species growing on artificial substrates. These communities generally showed stimulation of growth and some species composition changes in response to leachate concentrations of 3% v/v and higher. Concentrations of 15-20% v/v distillate inhibited the growth of stream periphyton. Periphyton exposed to distillates accumulated aliphatic hydrocarbons.

2) Short-term laboratory bottle tests with test species of algae generally showed growth inhibition with leachate and distillate concentrations of 5-20% v/v. However, when distillates were bubbled to remove volatile organic compounds, growth stimulation frequently was observed.

3) Three *in situ* experiments in a small lake were conducted with distillates at concentrations of 1, 5, and 20% v/v. Increases in algal biomass and bacteria populations in distillate-treated enclosures were observed in each of these tests. Although nearly all zooplankters were killed at distillate concentrations of 20%, reduced zooplankton grazing was probably not the cause of the observed increases in algal biomass.

Different samples of coal produced leachates and distillates which were highly variable in their characteristics. The stimulatory and toxic responses to coal distillates were apparently due to the presence of volatile organic compounds. Bioassays with these materials must be conducted and interpreted with care since many toxic compounds may escape to the atmosphere when test containers are bubbled, agitated, or are not tightly sealed. In the case of leachates, the reason for the differing algal responses in the

laboratory streams and the short-term bottle tests is not known. There is a need to critically examine different algal bioassay techniques to help resolve such inconsistencies in bioassay results.

This report was submitted in fulfillment of Grant No. R803932 by the University of Minnesota under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from July 15, 1975, to July 14, 1978, and work was completed as of July 14, 1978.

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Test algae for the bottle tests were supplied by William E. Miller, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency. The enclosure bottles for the field bioassays on Clearwater Lake were designed by Thomas E. Davis, Lake Superior Basin Studies Center, University of Minnesota, Duluth.

SECTION 1

INTRODUCTION

Increasing reliance on the use of coal as an energy source will necessarily involve the shipment and storage of extremely large quantities of this fuel. Much of the coal burned in the western and midwestern United States is low-sulfur coal derived from recently expanded mining activities in Montana, Wyoming, and North Dakota. For example, in Superior, Wisconsin, a new transshipment facility has been built on a 200-acre (81-hectare) site to help meet the energy needs of southeastern Michigan. Coal is shipped by rail from Decker, Montana, to the Superior facility where it is stored in open air holding piles. Eventually it is transferred to lake-going vessels and carried to power plants in Michigan. In all, approximately 200 million tons of Decker coal will be delivered to Michigan power plants, with shipments reaching 8 million tons per year by 1980.

Environmental hazards associated with the shipment and storage of this coal are largely unexplored. In spite of sophisticated dust suppression and water treatment systems, the movement of large quantities of coal will inevitably release coal dust into the atmosphere; and during times of high rainfall or snowmelt, runoff from coal storage areas may enter receiving bodies of water. Spontaneous heating within coal storage piles may also release volatile organic compounds which eventually enter the aquatic environment. Although recent work with marine algae indicates that low molecular weight aromatic hydrocarbons (benzenes, xylenes, naphthalenes) are the primary toxicants associated with fuel oils, no comparable studies have been conducted with coal.

The objectives of this study were to assess the effects of coal-derived contaminants on freshwater algae and to identify the types of compounds in coal leachates and distillates which stimulate or inhibit algal growth. Although coal for the study was obtained from the Superior transshipment facility, no attempt was made to provide a site-specific or impact study of this facility. Instead, algal bioassays were chosen to provide information of a more widely applicable nature and to compare the results of different bioassay procedures.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The results of algal bioassays with laboratory prepared coal leachates and distillates have indicated that uncontrolled runoff from coal storage piles may pose a hazard to the aquatic environment. At coal storage facilities leachates are produced as rainwater percolates through coal, while distillates are a likely result of spontaneous heating within coal piles. Both types of materials produced inhibition and stimulation of algal growth, the response depending on the concentrations of volatile organic compounds and coal particulates, the species and growth habit of the test algae, and the type of bioassay experiment. Zooplankters were more sensitive to coal distillates than phytoplankters, while bacterial growth was enhanced by distillates. Bioaccumulation of aliphatic compounds was observed in periphyton cells exposed to coal distillates.

The algal bioassays suggest that dissolved, volatile organic compounds and coal particulates are the most hazardous materials which may enter the aquatic environment from coal piles. Additional information is needed to quantify the amounts of these and other materials entering receiving waters from various types of coal storage facilities and to determine the extent of their bioaccumulation. In addition, more work is needed to resolve inconsistencies in the results obtained from different algal bioassay techniques.

SECTION 3

BIOASSAY TECHNIQUES

LABORATORY STREAMS

A laboratory stream facility was constructed to test the effects of coal leachates and distillates on periphyton communities growing on artificial substrates. This facility used raw Lake Superior water as "stream" water and as a source of periphyton organisms. Gerhart et al. (1977) have described in detail the initial design of the streams and also provided information concerning the replication of species composition, community structure, and biomass measurements. In some of the work presented here, several aspects of the design and operation of the streams differed from methods reported previously, and these are outlined below.

In August 1976, the stream facility was expanded to include twelve streams. Lighting was provided by lines of 40-watt Gro-Lux wide spectrum fluorescent lamps running parallel to the stream channels. Light intensity was 4,000 lux at the surface of the water; current speed was 16 cm/sec. The bottoms of the stream channels were insulated with foam sheeting.

In November 1976, the valves which had previously controlled lake water flow to the streams were replaced with capillary tubes in two secondary headboxes, each of which served six streams. These headboxes, polyethylene pails of 4-l capacity, were each fitted with six capillary tubes in their bases and an overflow tube which maintained the water level above the openings of the capillary tubes. The water from a capillary tube dripped into an open funnel (to prevent air locks) and flowed through polyethylene tubing to one of the streams. The flow in each tube was maintained at 16 ml/min by adjusting its height in the headbox. The headboxes and capillary tubes were blackened on the outside to prevent the growth of algae which might obstruct water flow. This arrangement allowed accurate control of low flows of incoming lake water, which in turn permitted higher concentrations of leachates and distillates to be tested. A second modification was the replacement of the unglazed porcelain growth substrates, used previously, with 10 x 10-cm quarry tile plates of fired clay.

A final procedural change was initiated in August 1977 and concerned the colonization of the substrates by periphyton organisms. In earlier experiments periphyton algae in the incoming lake water were allowed to extablish themselves naturally on the substrates. However, at low headbox flow rates, colonization by this method proved to be slow and erratic, leading to poor replication of experimental communities. To ensure an

adequate supply of viable cells to colonize the substrates at low headbox flows, the streams were seeded in the following manner. Nearshore periphyton communities were collected from Lake Superior, homogenized to form a slurry, and filtered through an 80-μ plankton net. On the first day of an experiment each stream was seeded with 100 ml of the slurry, added at the stream outlets. This technique resulted in rapid colonization and well-replicated communities. Species composition and community structure were found to be similar in the slurry and in the communities which developed on the substrates.

BOTTLE TESTS

Short-term (3-7 day) bottle tests were conducted in 300-ml Erlenmeyer flasks at 25-27°C. Culture volume was 100 ml. The flasks were maintained on a rotary shaker at approximately 80 rpm. Continuous illumination of 2,200-3,700 lux was provided by "cool-white" fluorescent lamps. Bioassays were conducted both in a synthetic nutrient medium (Environmental Protection Agency, 1971) and in phosphate-enriched Lake Superior water which had been filtered through Whatman GF-F glass fiber filters. In some cases the bottles were treated as batch cultures, with no additions during the course of an experiment. In other "replacement" tests the bottles were treated as semi-continuous cultures, with 50% of the culture volume removed and replaced daily with fresh medium and test additions. Tests were conducted in both foam and neoprene stoppered flasks.

The test algae were maintained in the synthetic nutrient medium, and test flasks were inoculated with 1 ml of exponentially growing stock culture. The algae were not washed prior to inoculation. The test algae included a local greenhouse strain of Chlorella and the following algae obtained from the EPA Corvallis Environmental Research Laboratory: Selenastrum capricornutum, Nitzschia palea, and Anabaena flos-aquae. All other procedures were those of the EPA Algal Assay Procedure (Environmental Protection Agency,1971).

FIELD BIOASSAYS

Three field bioassays with coal distillate were conducted on a small mesotrophic lake using in situ enclosures. Clearwater Lake, located 24 km north of Duluth, Minnesota, occupies a sheltered basin less than 0.1 km^2 in area and contains clear, unstained water. The lake has a maximum depth of 6 m and a secchi disk value of approximately 3 m. Total alkalinity is only 4 mg/l as $CaCO_3$, conductivity is approximately 31 μmhos/cm, and the pH ranges from 6.5-6.8. Several species of Dinobryon were the dominant phytoplankters when bioassays were conducted.

Eight 21-l Pyrex carboys were suspended individually from buoys at a depth of 1.5 m (Figure 1). The buoys were anchored on separate anchor lines in 4-5 m of water. Each carboy was fitted with a neoprene stopper held tightly in place with metal clamps (Figure 2). Two tubes passed through each stopper: an air exhaust/inlet tube and a sampling tube. A hand pump was fitted with polypropylene tubing and PVC elbows at each end and used

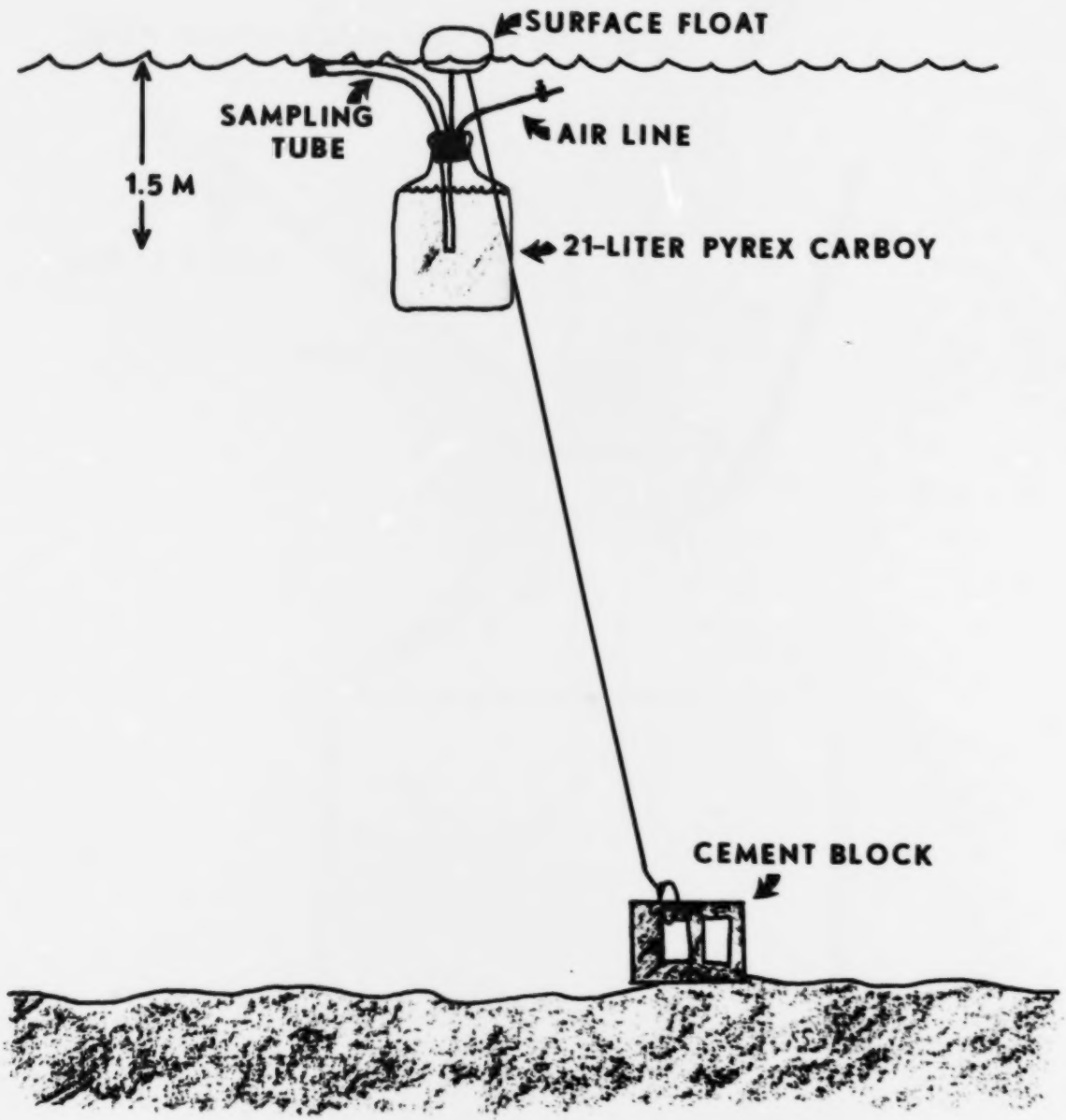

Figure 1. Field bioassay enclosure system.

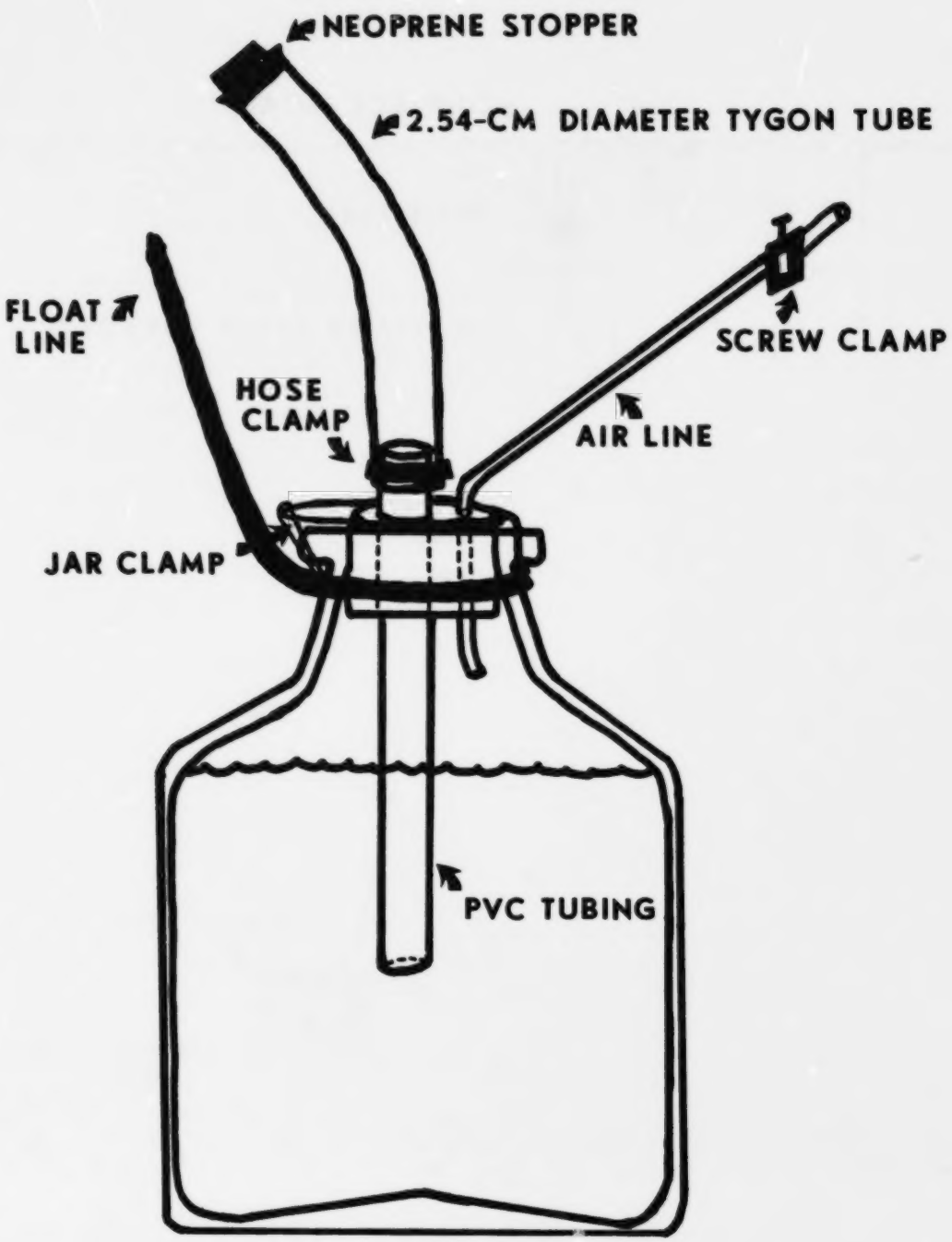

Figure 2. Detail of field enclosure bottle.

both to withdraw and return water to the carboys. For sampling, the elbow on the output end of the pump was inserted into the sampling tube of the carboy, the clamp on the air exhaust/inlet tube was opened, and 15-20 pump strokes of air were pumped into the carboy to mix its contents thoroughly. The pump was then immediately reversed, and 18% of the carboy's contents was removed. This water was used for sample water for all analyses. A standard volume of distilled water (control carboys) or coal distillate (treatment carboys) was then poured into the carboy through the sampling tube, the pump was again reversed, and lake water was pumped from a depth of 1.5 m into the carboy to restore the original volume (18 liters). This scheme permitted all sampling operations to take place without removing the carboys from the Lake. Samples were collected three days per week. In the first two bioassays (September and October, 1977) 18% of the carboy volume was replaced daily with fresh lake water and the appropriate volume of distilled water or coal distillate. In the third bioassay (June 1978) 33% of the carboy volume was replaced three times per week when the bottles were sampled.

BIOMASS MEASUREMENTS

Chlorophyll *a* analyses were conducted *in vivo* with a Turner Model 111 fluorometer or on samples filtered through Whatman GF/F glass fiber filters and extracted in 90% acetone. In the latter case, either fluorometric determinations or spectrophotometric measurements at 663 nm were employed. All chlorophyll *a* and phaeopigment methods were those of Strickland and Parsons (1968). Fluorometric measurements of *in vivo* samples before and after the addition of 10 μM DCMU were used to estimate photosynthetic capacity and algal viability (Slovacek and Hannan, 1977; Samuelsson et al., 1978).

Ash-free dry weight analyses of periphyton and plate counts of bacteria populations were performed using methods described by the American Public Health Association (1975). ATP was extracted from algal samples with boiling Tris buffer after filtration onto glass fiber filters (Holm-Hansen and Booth, 1966; Rudd and Hamilton, 1973). The extracted ATP was frozen until analysis with a DuPont Bioluminescence Photometer. The cellular organic carbon content of algal samples was found by multiplying ATP concentrations by 286 (Stadelmann, 1974).

Cell counts of periphyton diatoms were made by filtering a known volume of sample through a 25-mm membrane filter (0.45 μm) and then washing the filter with 5 ml of 50% ethanol and 5 ml of absolute ethanol. After drying, the filter was mounted on a glass slide in creosote and the edges of coverslip were sealed with melted paraffin. Generally, 8,000 cells were counted per sample. Samples for counts of algae other than diatoms were preserved in 1% acid Lugol's solution and counted using an inverted microscope technique (Vollenweider, 1974).

CHEMICAL ANALYSES

Methods used in the chemical analysis of water samples and coal leachates and distillates are listed in Table 1. For tissue analysis of

algal cells, periphyton samples from stream substrates were centrifuged and dried at 50°C to constant weight. An 0.8000 g portion was removed, mixed with 10 g sodium sulfate, and Soxhlet extracted for 5 h with 200 ml 50:50 methylene chloride/hexane. The extract was concentrated to dryness, resuspended in hexane, and concentrated to 10 ml. One ml was then removed for

TABLE 1. METHODS FOR CHEMICAL ANALYSES

Analysis	Method	Reference
Major cations	Flame atomic absorption	-
Trace metals	Flameless atomic absorption	-
Chloride	Ferric thiocyanate spectrophotometric	American Society of Testing and Materials, 1976
Reactive phosphorus	Ascorbic acid/molybdate	American Public Health Association, 1975
Total phosphorus	Persulfate digestion	"
Nitrate nitrogen	UV spectrophotometric	"
Ammonium	Nesslerization	"
Dissolved organic nitrogen	Sulfuric acid digestion	"
Sulfate	Turbidimetric	"
Total alkalinity	Bromcresol green/methyl red indicator	"
Turbidity	Nephelometric	"
Total organic carbon	Combustion, IR analysis	"
Phenol	4-aminoantipyrene spectrophotometric	"
Chemical oxygen demand	Dichromate reflux	"
Organics	UV analysis	Mrkva, 1969; Mattson et al., 1974
Organics	Gas chromatography	See text
Complexing capacity	Modified	Chau and Lum-Shu-Chan, 1974

lipid analysis, and 5 ml were transferred to a silicic acid column. Aliphatics, aromatics, and polar compounds were collected with 12-ml washes using hexane, benzene, and methanol, respectively. The benzene fraction was transferred to a Florisil column and eluted with 50 ml hexane to remove lipids. The hexane and benzene fractions were concentrated in a Kuderna-Danish evaporator, and 4 µl fractions were injected into a Varian 1700 gas chromatograph (GC).

Coal distillate and leachate were also analyzed by GC. A 1-l sample was extracted for 2 h with 50 ml of 50:50 methylene chloride/hexane. The solvent layer was removed, run through sodium sulfate, and concentrated in a Kuderna-Danish evaporator. The 1-ml concentrate was then added to a silicic acid column and eluted with hexane, benzene, and methylene chloride. GC analysis was as described for algal tissue except that no Florisil cleanup was used. Blanks were performed on the silicic acid column, and no contamination was found. A simplified method was used to analyze 2-l samples of leachate and unbubbled and bubbled distillate. These samples were extracted with 40 ml methylene chloride for 2 h. The solvent layer was removed, and 5 µl sub-samples were injected directly into the GC.

For all samples the GC was equipped with a flame ionization detector (F.I.D.) and a 2 m x 2 mm i.d. glass column packed with 3% OV-101 on 80-100 mesh Gas Chrom Q (injector T = 250°, detector T = 300°C). The column was programmed from 100-250°C at 4°/min for the tissue analyses and 1-l distillate sample and from 60-200°C at 4°/min for the 2-l leachate and unbubbled and bubbled distillate samples.

SECTION 4

PREPARATION AND CHARACTERISTICS OF COAL LEACHATES AND DISTILLATES

All coal for the preparation of leachates and distillates was collected from storage piles at the transshipment facility in Superior, Wisconsin. Collections were made several times a year in clean polyethylene bags. Because of the extremely large coal storage area at the facility and the continuous activity of bulldozers and conveyor belts, it was not possible to obtain random or representative samples from the piles. Thus, the coal samples used in this study were by no means uniform in their physical and chemical characteristics.

COAL LEACHATES

Coal leachates for heavy metal analyses were prepared by pumping double-distilled, deionized water through samples of ground coal which had been sieved to remove particles smaller than 0.25 mm and greater than 2.0 mm diameter. A Wiley mill and stainless steel sieves were used to grind and sieve the coal. Coal samples were held in a plexiglass tube while a peristaltic pump circulated the water from a polyethylene reservoir through the sample and back to the reservoir. The coal was leached continuously for 65 h, and the resulting leachate was filtered through washed membrane filters (0.45 μ) to remove suspended coal particles. Leachates were prepared using coal/water weight ratios of 0.004 and 0.016. These leachates were quite low in concentrations of trace metals and other ions (Table 2).

All other leachates and all leachates used in bioassays were prepared using a coal/water weight ratio of 0.08. Ground, unsieved coal and double-distilled, deionized water were added to a polyethylene carboy and mixed continuously with an electric stirrer for at least 24 h. For most stream bioassays this leachate was centrifuged in a continous-flow centrifuge (Sorvall SS-3) at 12,000-14,000 rpm. In several stream bioassays the centrifuged leachate was filtered through Whatman GF/F glass fiber filters, and for most bottle tests the leachate was not centrifuged but was filtered through membrane filters (0.45 μ). Characteristics of these leachates are reported in Table 3. In addition, a single measurement of complexing capacity indicated that 1 l of leachate will complex 128 μg of copper ion.

Considerable variation in leachate characteristics was found among leachates prepared from different batches of coal. However, most leachates appear to contain relatively high concentrations of sodium and sulfate ions

TABLE 2. CONCENTRATIONS OF TRACE METALS AND OTHER IONS IN COAL LEACHATES*

Parameter	0.004 w/w leachate	0.016 w/w leachate
Conductivity (μmhos/cm)	15-17	28-36
pH	7.0-7.4	7.4-7.7
Reactive phosphorus	-	<1
Nitrate nitrogen	-	75-166
Lead	<0.5	<0.5
Zinc	0.4-2.1	0.6-2.0
Manganese	<0.2	<0.2-0.5
Copper	0.1-0.6	<0.2-0.6
Cadmium	<0.02	0.05-0.08
Chromium	0.2	<0.5
Iron	1.7-2.5	-
Nickel	-	<2.0

*All element concentrations are in μg/1.

and small but significant quantities of dissolved organic and particulate phosphorus (Table 3). The pH of leachates was always between 7.0 and 8.0.

Direct GC analysis of a methylene chloride extraction of a 2-l sample of membrane filtered leachate showed that few non-polar organic compounds were present and that concentrations of these compounds were extremely low (Figure 3). Bubbling leachates with filtered air was not effective in reducing the concentrations of organic compounds. The UV absorption spectrum for filtered leachates showed gradually decreasing absorbance values as the wavelength was increased from 210 nm. There were no absorbance peaks in the UV spectrum. It is likely that absorbances in the UV were due largely to the presence of extremely fine particles (<0.45 μ diameter) in filtered leachates. Similarly, most of the total organic carbon (TOC) content of filtered leachates was probably due to these particulates rather than to the presence of dissolved organic compounds. TOC values for membrane filtered leachates averaged 19 mg/l, while a single measurement of a centrifuged (unfiltered) leachate sample yielded a TOC value of 78 mg/l.

COAL DISTILLATES

All coal distillate was prepared by distilling 4 l of double-distilled, deionized water containing 1,000 g ground, unsieved coal and collecting the condensate. Coal distillate prepared in this manner had a conductivity of 5-30 μmhos/cm, a pH of 4.5-5.5, and a total phosphorus content of approximately 1 μg/l. A single batch of distillate was found to have a phenol concentration of 0.24 mg/l.

The organic carbon content of coal distillates varied considerably with different coal samples. Chemical oxygen demand (COD) values as low as 15 and as high as 127 mg/l were recorded, while TOC ranged from 5-34 mg/l. The UV absorption spectrum for distillates had a minimum at 236 nm and a maximum at 254-256 nm. Below 236 nm, absorbance increased rapidly. Most of the organic compounds present were highly volatile. When coal distillate was bubbled with filtered air in 250-ml Erlenmeyer flasks, UV absorbances decreased significantly, and this decrease was greater at lower wavelengths than at 254 nm (Table 4). Swirling flasks of distillate on a mechanical shaker and warming the samples on a hotplate were also observed to reduce UV absorbances. In addition, some loss of organic compounds occurred when distillate was stored, even when tightly capped and refrigerated. Bubbling also affected distillate pH and conductivity (Table 5). The pH of distillate was nearly two units higher after bubbling for 30 min, while the conductivity dropped to approximately half that of the unbubbled sample.

Fractionation and GC analysis of a 1-l sample of coal distillate indicated that the predominant organics were aliphatics, with lesser concentrations of aromatic and polar compounds (Figure 4). Direct GC analysis of a methylene chloride extraction of a 2-l distillate sample showed the predominance of early-eluting (volatile) organic compounds. Most compounds appeared on the chromatogram before the temperature reached 150°C during the GC temperature program. GC analysis of bubbled distillate showed decreased concentrations of many non-polar organic compounds relative to unbubbled distil-

late (Figure 5).

TABLE 3. CHARACTERISTICS OF 0.08 W/W COAL LEACHATES*

Parameter	Approximate Range	Approximate Mean
pH	7.0-7.7	7.4
Conductivity (μmhos/cm)	70-350	200
Suspended Solids	8-140	highly variable
Turbidity (NTU)	20-65	52
Alkalinity (as $CaCO_3$)	5-25	15
Calcium	0.1-0.3	0.2
Magnesium	0.05-0.15	0.10
Sodium	30-90	53
Potassium	0.7-0.8	0.8
Chloride	2.4-3.0	2.7
Sulfate	40-120	63
Nitrate nitrogen	0.02-0.12	0.07
Ammonium nitrogen	-	0.15
Dissolved organic nitrogen	-	1.35
Reactive phosphorus	<0.001-0.008	0.001
Total dissolved phosphorus	0.01-0.10	0.05
Total phosphorus	0.02-0.12	0.07
Phenols	-	0.16
Total Organic Carbon	13-27	19

*All concentrations are mg/l. Centrifuged leachate samples were filtered through glass fiber or membrane filters for all analyses except suspended solids, turbidity, and total phosphorus. A dash (-) in the "Range" column indicates that only one sample was analyzed.

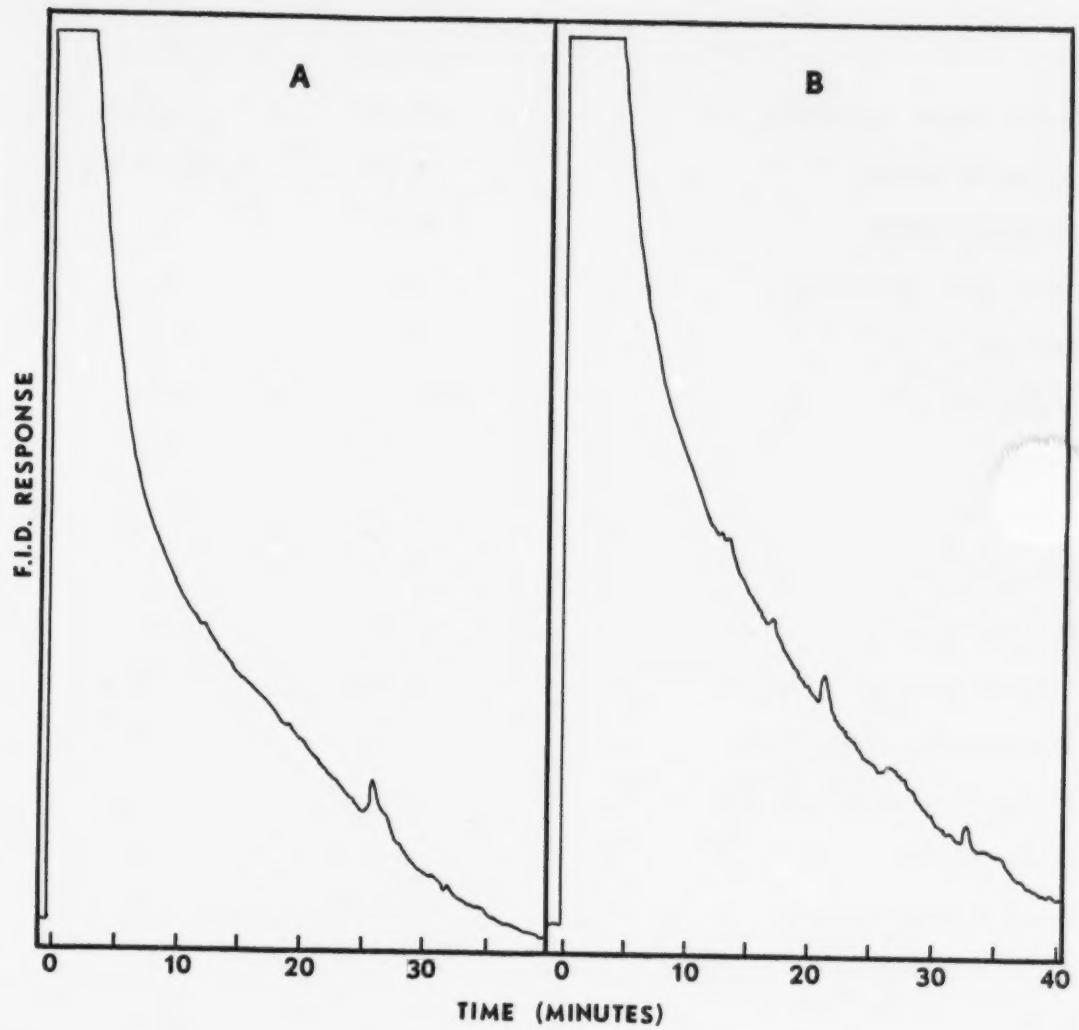

Figure 3. GC analysis of a 2-l sample of membrane filtered leachate. A- Membrane filtered distilled water blank; B- Membrane filtered leachate.

TABLE 4. ABSORBANCE VALUES (1-CM CELLS) FOR COAL DISTILLATE BEFORE AND AFTER VARIOUS PERIODS OF BUBBLING

Wavelength (nm)	Bubbling Time (min) 0	0.5	1.0	3.0	5.0	15.0
200	0.780	0.753	0.713	0.650	0.602	0.504
220	.679	.663	.633	.587	.540	.425
230	.354	.346	.333	.301	.285	.220
240	.297	.291	.286	.273	.252	.210
254	.496	.487	.473	.455	.423	.342
546	.002	.004	.003	.003	.002	.006

TABLE 5. EFFECTS OF A 30-MIN BUBBLING PERIOD ON COAL DISTILLATE pH AND CONDUCTIVITY

Sample	pH Initial	pH Final	Conductivity (μmhos/cm) Initial	Conductivity (μmhos/cm) Final
1	4.9	6.5	14.8	7.9
2	4.6	6.4	16.7	7.2

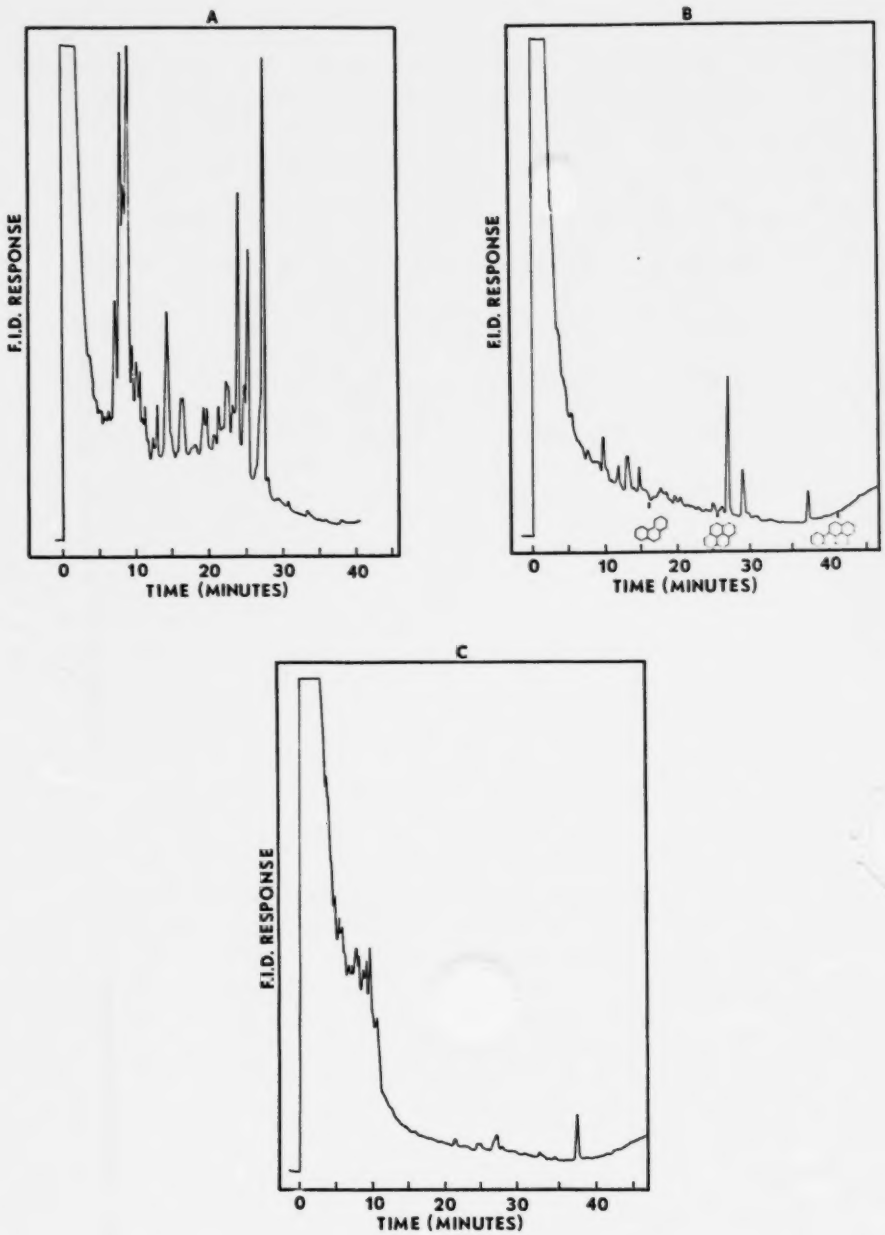

Figure 4. GC fractionation of coal distillate. A- Aliphatic fraction; B- Aromatic fraction; C- Polar fraction.

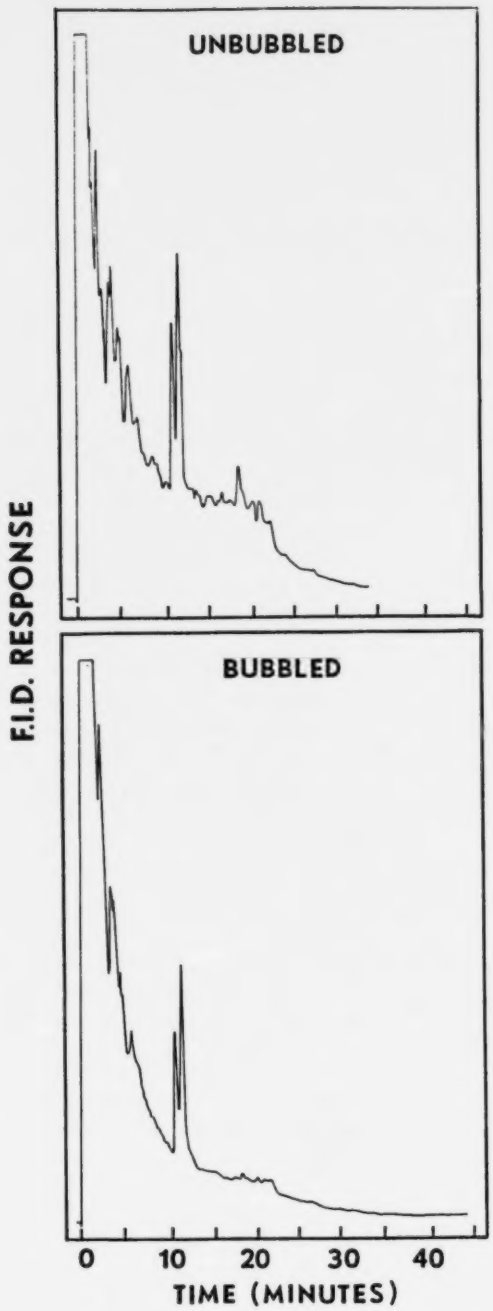

Figure 5. GC analysis of 2-1 samples of unbubbled and bubbled distillate. Bubbling time was 60 min.

SECTION 5

EXPERIMENTAL PROCEDURES AND RESULTS

COAL LEACHATES

Laboratory Streams

Early experiments with extremely low concentrations of coal leachate showed no effects on periphyton growth in the laboratory streams (Gerhart et al., 1977). However, three experiments performed with 3.2% v/v leachate suggested some stimulation of periphyton growth by leachates. These tests were conducted at water temperatures ranging from 11-16°C and lasted from 18-24 days. The first was conducted in June, the second in July, and the third in September 1976.

With few exceptions, chlorophyll *a* concentrations were higher in leachate streams than in controls (Figure 6). When cell counts were performed, these showed increases in diatom numbers in leachate streams (Figure 7). In the September experiment (Figure 7B) a species dominance shift was observed in leachate streams from *Achnanthes microcephala* to *Synedra* spp. This experiment also included a treatment of leachate plus 1 mg/l Cu^{++} to test the possibility that coal particles settling on the substrates in leachate streams might interfere with chlorophyll determinations. No significant interference was found (Figure 6C).

Visual differences between treatment and control streams were evident in these experiments. Substrates in leachate streams had a definite dark brown color relative to controls, due both to heavier algal growth and sedimentation of coal particles.

Experiments conducted with higher concentrations of coal leachates also indicated growth stimulation. One experiment was conducted with 17% v/v leachate in December 1976 and January 1977 when stream water temperatures were 8-10°C. The conductivity of the leachate used in this run averaged 239 µmhos/cm, and leachate streams had somewhat higher conductivities (avg. = 111 µmhos/cm) than control streams (avg. = 95 µmhos/cm). The leachate had a suspended solids concentration of approximately 134 mg/l. Growth was initially slow in all streams, and on the 23rd day of the experiment the streams were enriched on a continuing basis with 5 µg/l PO_4-P. Growth was rapid following this enrichment, with leachate streams showing higher values for chlorophyll *a* and ash-free dry weight than controls (Figure 8). Changes in species composition were also evident (Figure 9). Leachate streams showed decreased importance of *Nitzschia palea* and *Fragilaria* spp.

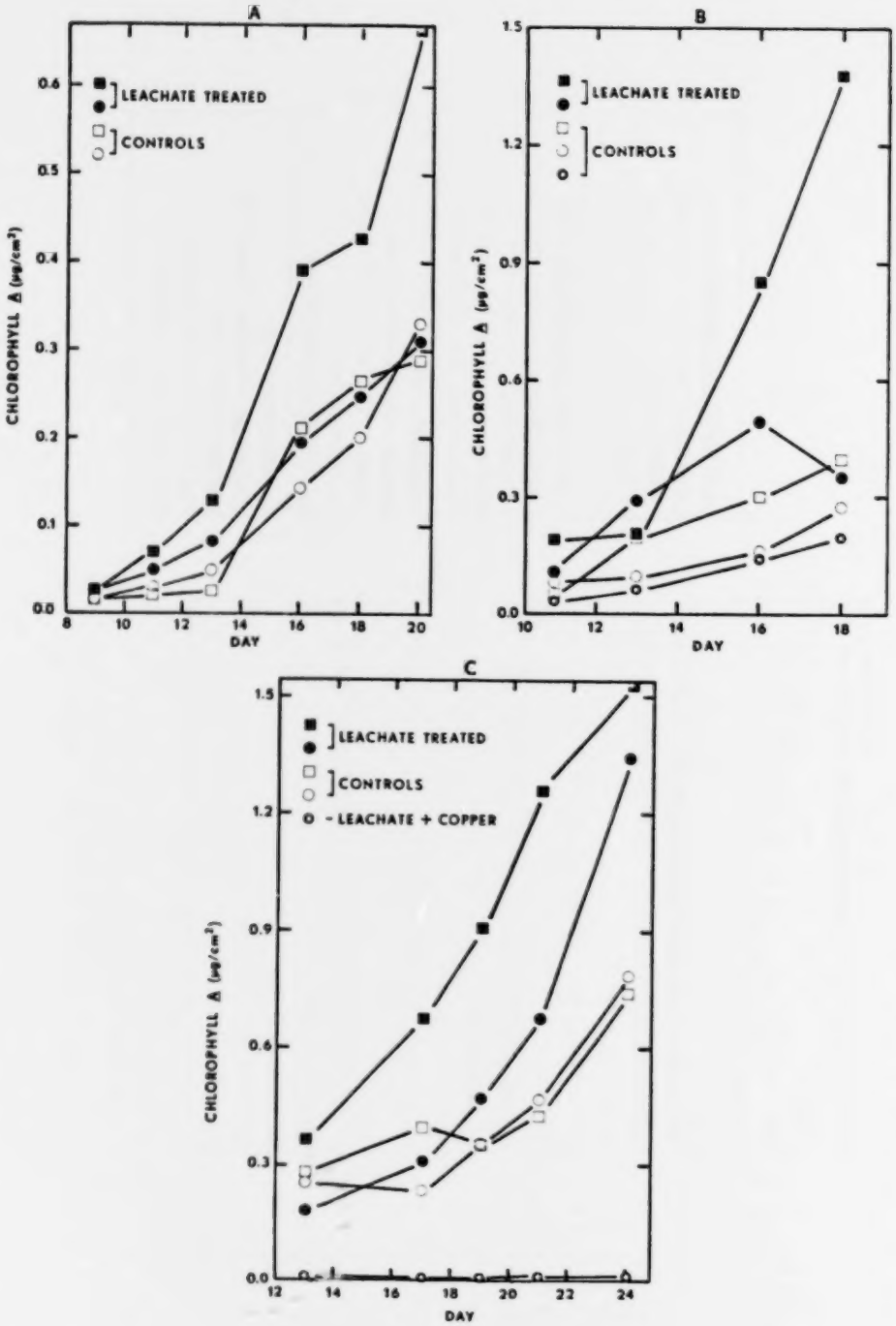

Figure 6. Effects of 3.2% v/v leachate on laboratory stream periphyton. A- June 1976; B- July 1976; C- September 1976.

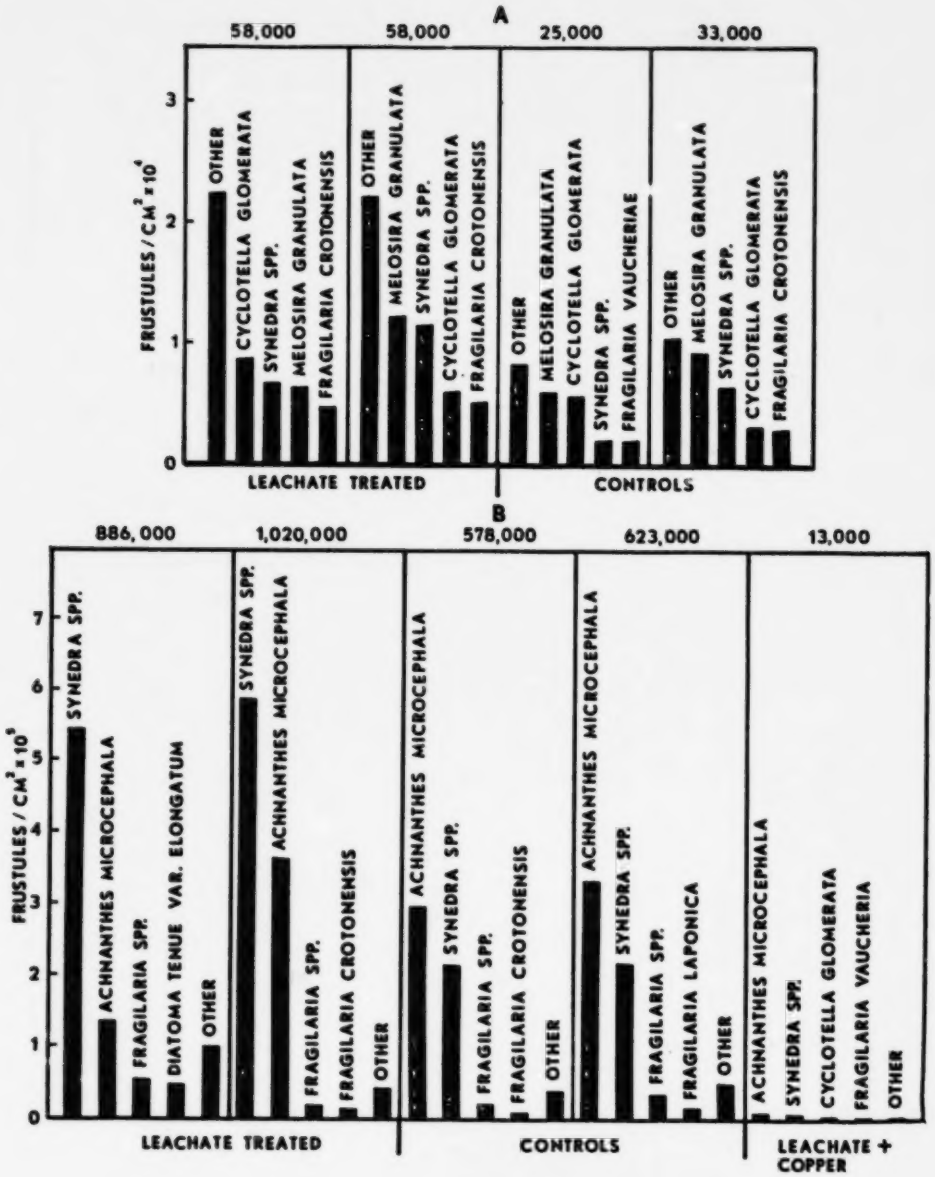

Figure 7. Effects of 3.2% v/v leachate on species composition and abundance of laboratory stream periphyton. A- June 1976, day 13; B- September 1976, day 24. Numbers at top of figures indicate total frustule counts.

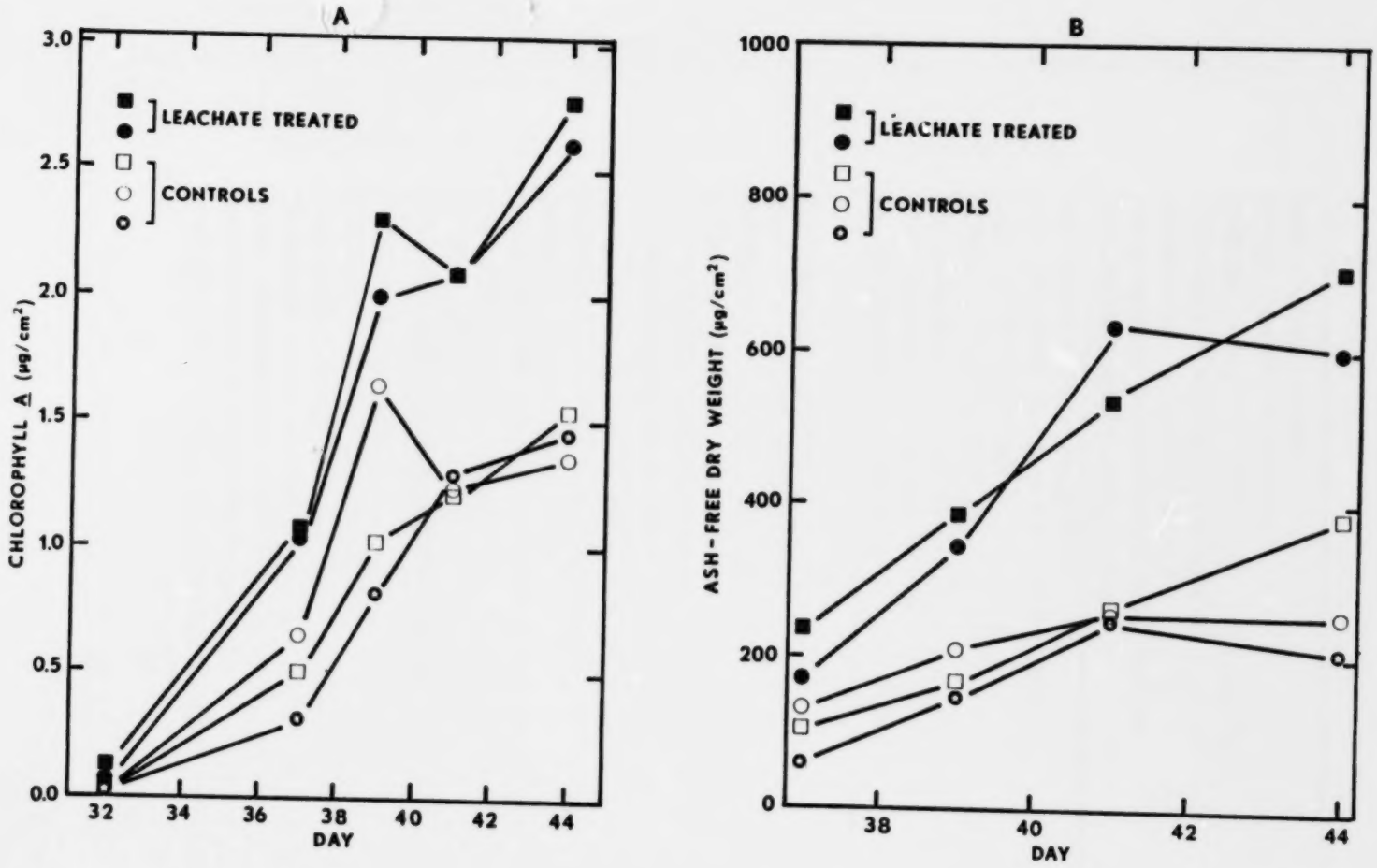

Figure 8. Effects of 17% v/v leachate on laboratory stream periphyton, December 1976 and January 1977. A- Chlorophyll a; B- Ash-free dry weight.

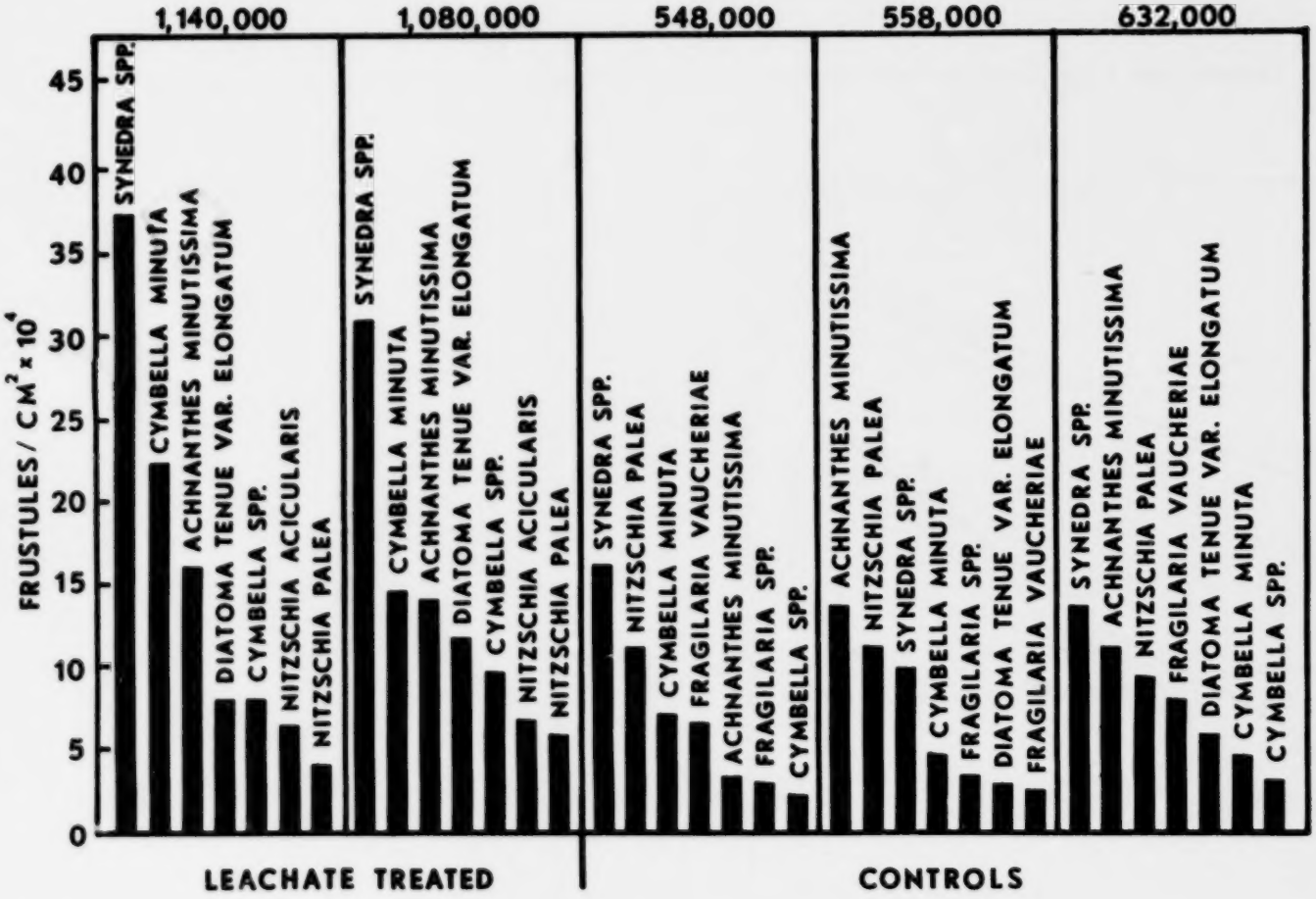

Figure 9. Effects of 17% v/v leachate on species composition and abundance of laboratory stream periphyton, January 1977, day 41. Numbers at top of figure indicate total frustule counts.

and increased importance of Cymbella spp. and Nitzschia acicularis. These trends were confirmed by cell counts on day 37. A statistical analysis (F-test and t-test using log transformations) indicated that the means of all these groups except Nitzschia palea were significantly different at the 95% level in treatment and control streams. The fact that Nitzschia palea was consistently less abundant in leachate streams on two sampling days makes it seem likely that this species too responded to the presence of leachate.

A second experiment with 17% v/v leachate was conducted in September 1977. To be certain that stimulatory effects observed previously were not a consequence of large amounts of particulates in coal leachate, the centrifuged leachates used in this experiment were filtered through Whatman GF/F glass fiber filters, thus reducing the concentration of suspended solids to approximately 22 mg/l. Stream water temperatures were 13-15°C, and all streams were enriched with 12 µg/l PO_4-P. The conductivity of control streams averaged 94 µmhos/cm, while leachate streams averaged 102 µmhos/cm. Chlorophyll a and ash-free dry weight analyses again indicated higher periphyton biomass in leachate streams (Figure 10). On day 17 two of the treatment streams showed higher frustule counts than the control streams, but differences in total counts were not significant (Figure 11). Non-diatoms were not counted. Cymbella minuta var. minuta was significantly more abundant in leachate than control streams; the reverse was true of Tabellaria fenestrata.

Several generalizations may be made regarding the stream bioassays with coal leachates. First, the pH of treatment streams was never significantly different from control streams. The pH of coal leachates was generally between 7.5 and 8.0, and this range is nearly identical to the pH range for Lake Superior water. Second, while dominance shifts and changes in algal species composition were observed in leachate streams, significant shifts in community structure (determined by plotting log-normal curves) were never detected. Third, total and total dissolved phosphorus concentrations were usually greater in leachate than control streams. Soluble reactive phosphorus concentrations, however, were not consistently greater in leachate streams.

Bottle Tests

Beginning in September 1977, a total of 11 small-volume bottle tests of the effects of coal leachates were conducted using test algal species. The control bottles in these experiments received volumes of double-distilled, deionized water equivalent to the test volumes of leachate. Most of the tests were conducted with membrane filtered (0.45 µ) leachates having turbidities of 3-14 NTU.

Three batch culture tests with centrifuged and membrane filtered leachates employed the alga Selenastrum capricornutum growing in synthetic nutrient medium (Environmental Protection Agency, 1971). Light intensity was 2,200 lux. At a concentration of 20% v/v both centrifuged and filtered leachates inhibited growth, measured by haemocytometer counts of algal cells (Figure 12). Centrifuged leachates contained greater amounts of coal

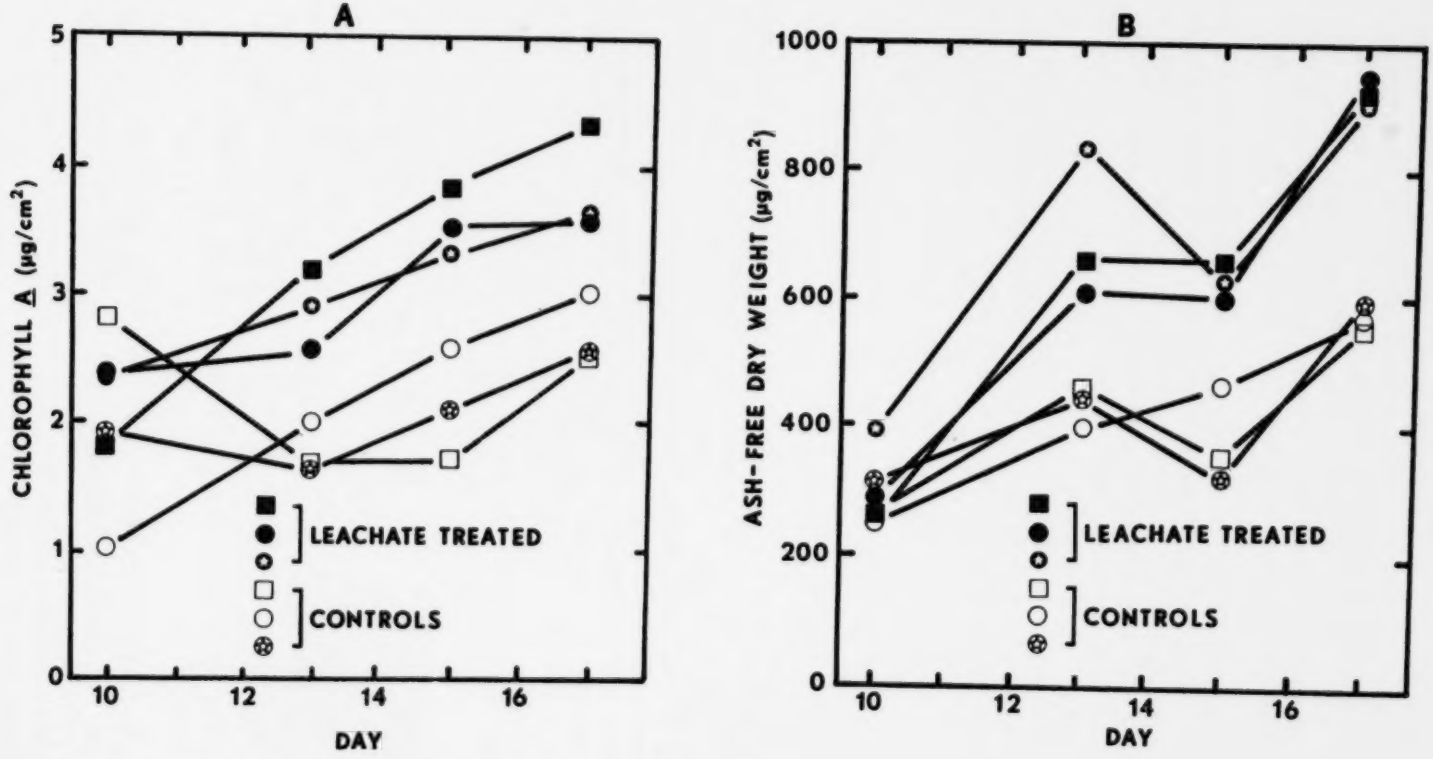

Figure 10. Effects of 17% v/v leachate on laboratory stream periphyton, September 1977. A- Chlorophyll a; B- Ash-free dry weight.

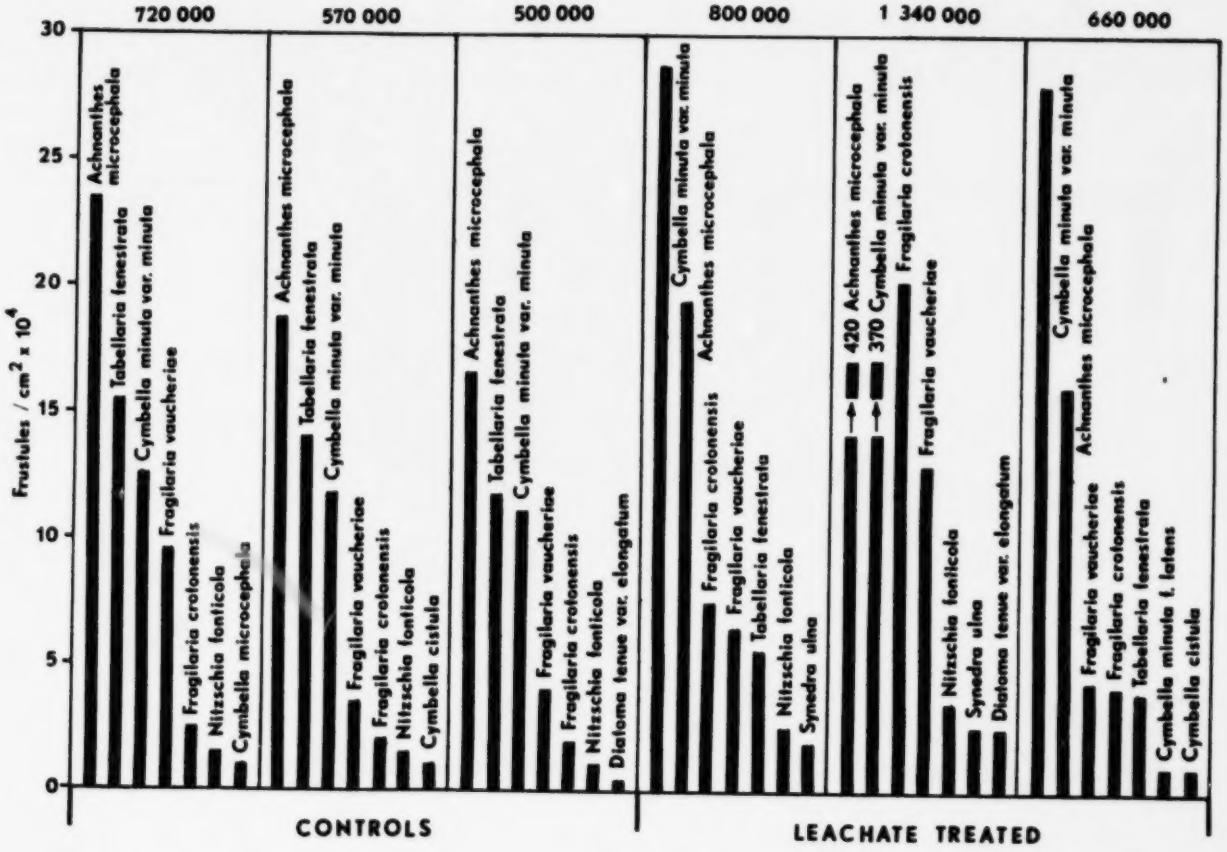

Figure 11. Effects of 17% v/v leachate on species composition and abundance of laboratory stream periphyton, September 1977, day 17. Numbers at top of figure indicate total frustule counts.

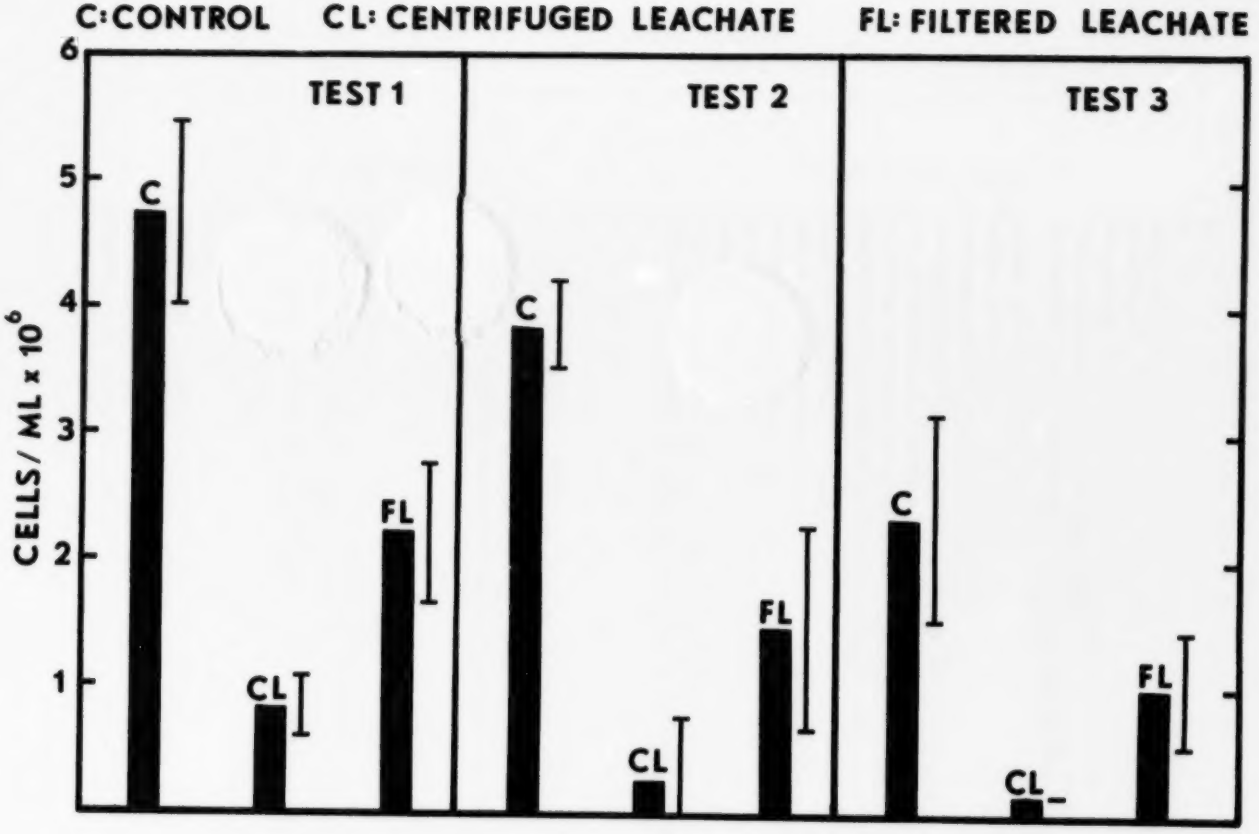

Figure 12. Effects of 20% v/v leachate on the growth of Selenastrum capricornutum in synthetic nutrient medium in batch cultures, day 6. Each treatment had 6 replicate flasks. Error bars indicate one standard deviation.

particulates and were more inhibitory than filtered leachates. The conductivities of centrifuged and filtered leachates were similar. Algae exposed to leachates exhibited abnormal cell structure when viewed under the light microscope.

Two 50%/day replacement experiments with 20% v/v membrane filtered leachate were conducted with Selenastrum growing in enriched Lake Superior water. Phosphorus enrichment of the lake water was 5, 20, or 200 µg/l PO_4-P. Light intensity in these and subsequent experiments was 3,700 lux. Fifty percent of the culture volume was replaced daily, and chlorophyll a was determined by in vivo fluorescence measurements after the addition of DCMU. The percent increase in fluorescence after the addition of DCMU was used as a measure of photosynthetic capacity. Leachates were prepared from two different coal samples, originating in different strip mines. The conductivity of the leachate used in the first test was 76 µmhos/cm, while that in the second test was 275 µmhos/cm. In every case leachate cultures achieved much lower chlorophyll levels than control cultures (Figure 13 and 14). Only slightly less inhibition was observed in cultures receiving the higher P additions. Percent increases in fluorescence after DCMU addition also indicated a reduction of photosynthetic capacity in leachate cultures. This trend was especially pronounced in the second test.

Two replacement tests were conducted simultaneously with v/v concentrations of 5, 10, and 20% filtered leachate. The test alga was Selenastrum capricornutum in the first test and Nitzschia palea in the second. The culture medium was filtered lake water enriched with 200 µg/l PO_4-P. Leachate conductivity was 143 µmhos/cm; turbidity was 11-12 NTU. Growth inhibition was observed at all concentrations with Selenastrum and at the 10 and 20% concentrations with Nitzschia (Figure 15). Differences in photosynthetic capacity between treatment and control flasks were generally not significant in these tests.

Phosphorus enriched (200 µg/l PO_4-P) lake water was also used as the growth medium for four batch tests with 20% v/v filtered leachate. When leachates having conductivities of 103-132 µmhos/cm and turbidities of 3-5 NTU were tested, no significant growth inhibition of Selenastrum or Nitzschia was observed (Figure 16). Anabaena flos-aquae may have been inhibited slightly by this treatment. However, a leachate having a conductivity of 71 µmhos/cm and a turbidity of 14 NTU did inhibit the growth of Nitzschia (Figure 17). Significant differences in photosynthetic capacity between treatment and control flasks were not observed in any of these tests.

COAL DISTILLATES

Laboratory Streams

Several stream bioassays were performed in which periphyton communities were exposed to 10-20% v/v coal distillate for periods of 1-10 days. All of these experiments suggested some inhibition of periphyton growth by distillates, but the results were usually inconclusive. One such experiment was conducted in February and March, 1977, primarily to obtain algal cells for

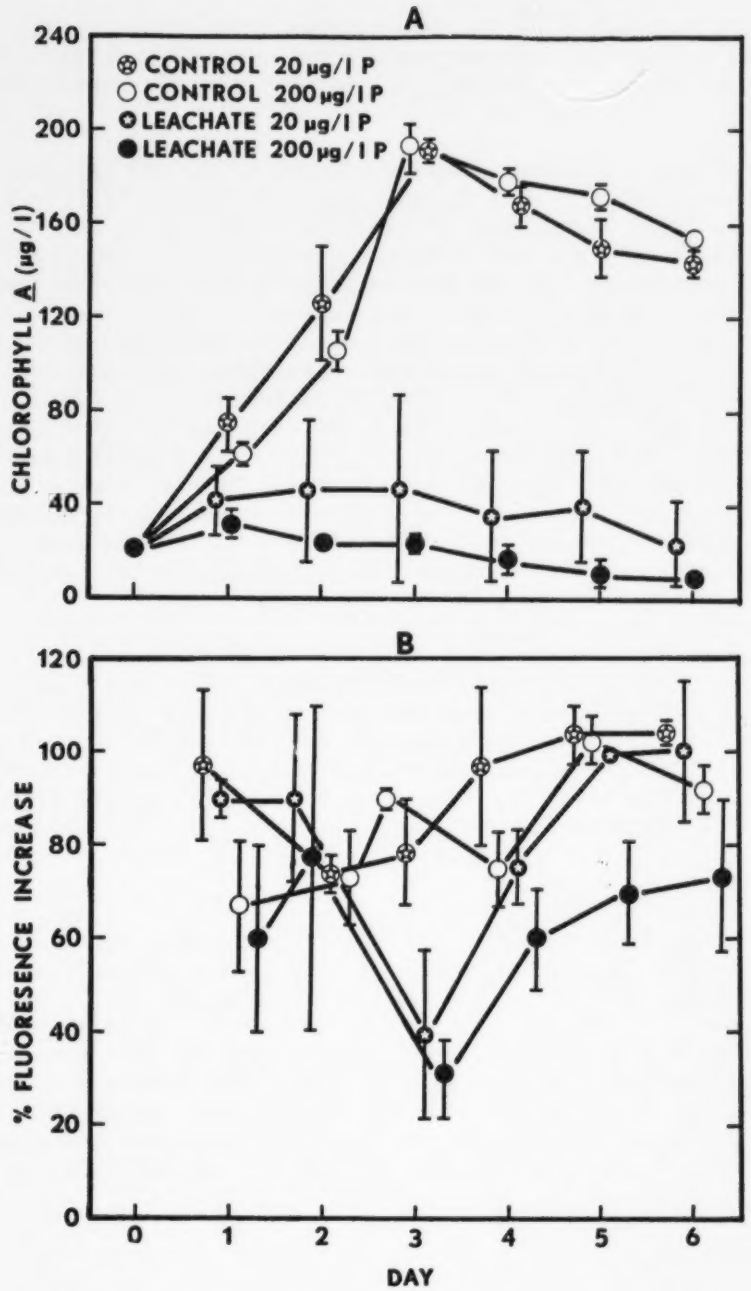

Figure 13. Effects of 20% v/v leachate on the growth of Selenastrum capricornutum in Lake Superior water in 50%/day replacement cultures, Test 1. Each treatment had 4 replicate flasks. Error bars indicate one standard deviation. No error bars are shown where the bar is smaller than the symbol. Leachate conductivity was 76 µmhos/cm. A- Chlorophyll a; B- Photosynthetic capacity.

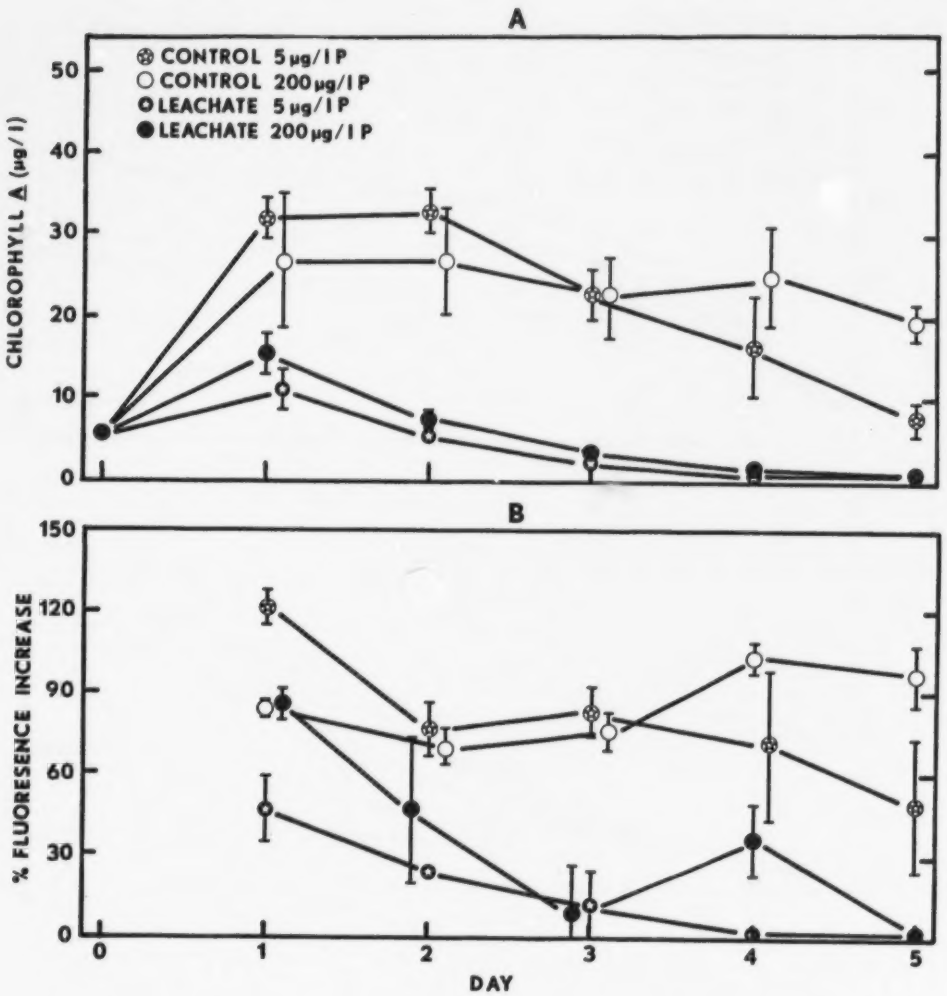

Figure 14. Effects of 20% v/v leachate on the growth of Selenastrum capricornutum in Lake Superior water in 50%/day replacement cultures, Test 2. Each treatment had 4 replicate flasks. Error bars indicate one standard deviation. No error bars are shown where the bar is smaller than the symbol. Leachate conductivity was 275 µmhos/cm. A- Chlorophyll a; B- Photosynthetic capacity.

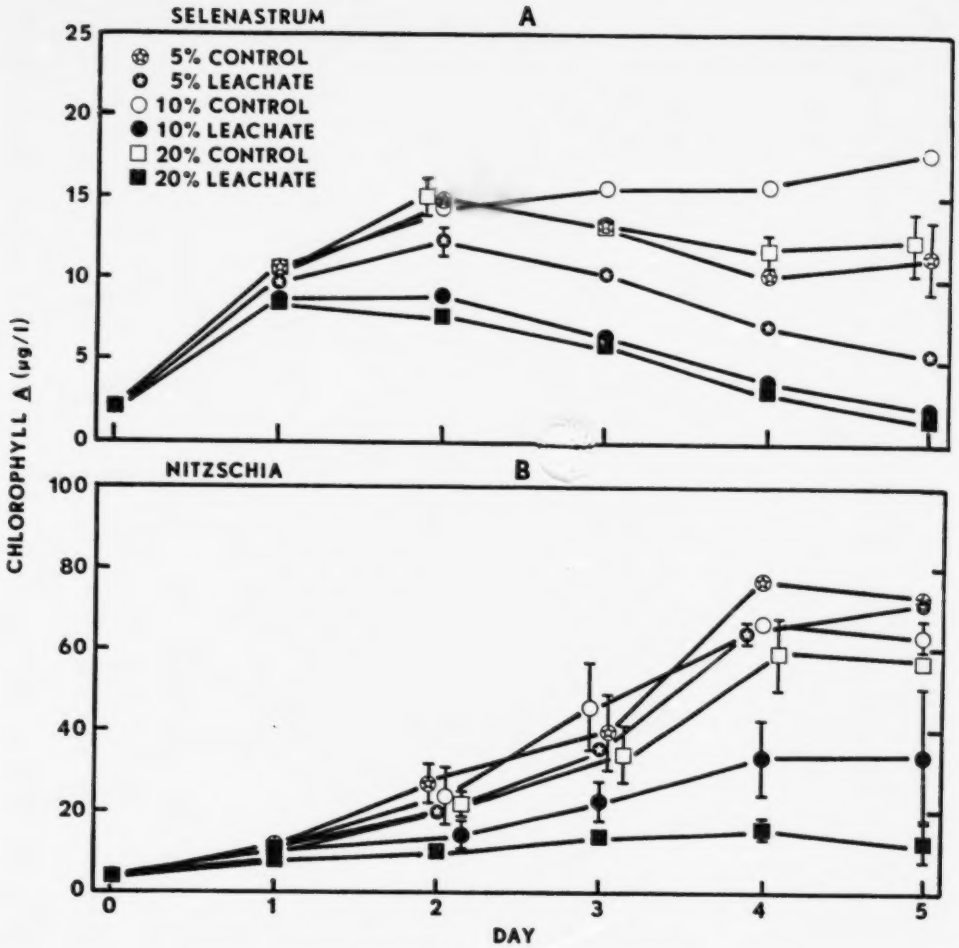

Figure 15. Effects of 5%, 10%, and 20% v/v leachate on the growth of Selenastrum capricornutum (A) and Nitzschia palea (B) in Lake Superior water in 50%/day replacement cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. No error bars are shown where the bar is smaller than the symbol. Leachate conductivity was 143 µmhos/cm; turbidity was 11-12 NTU.

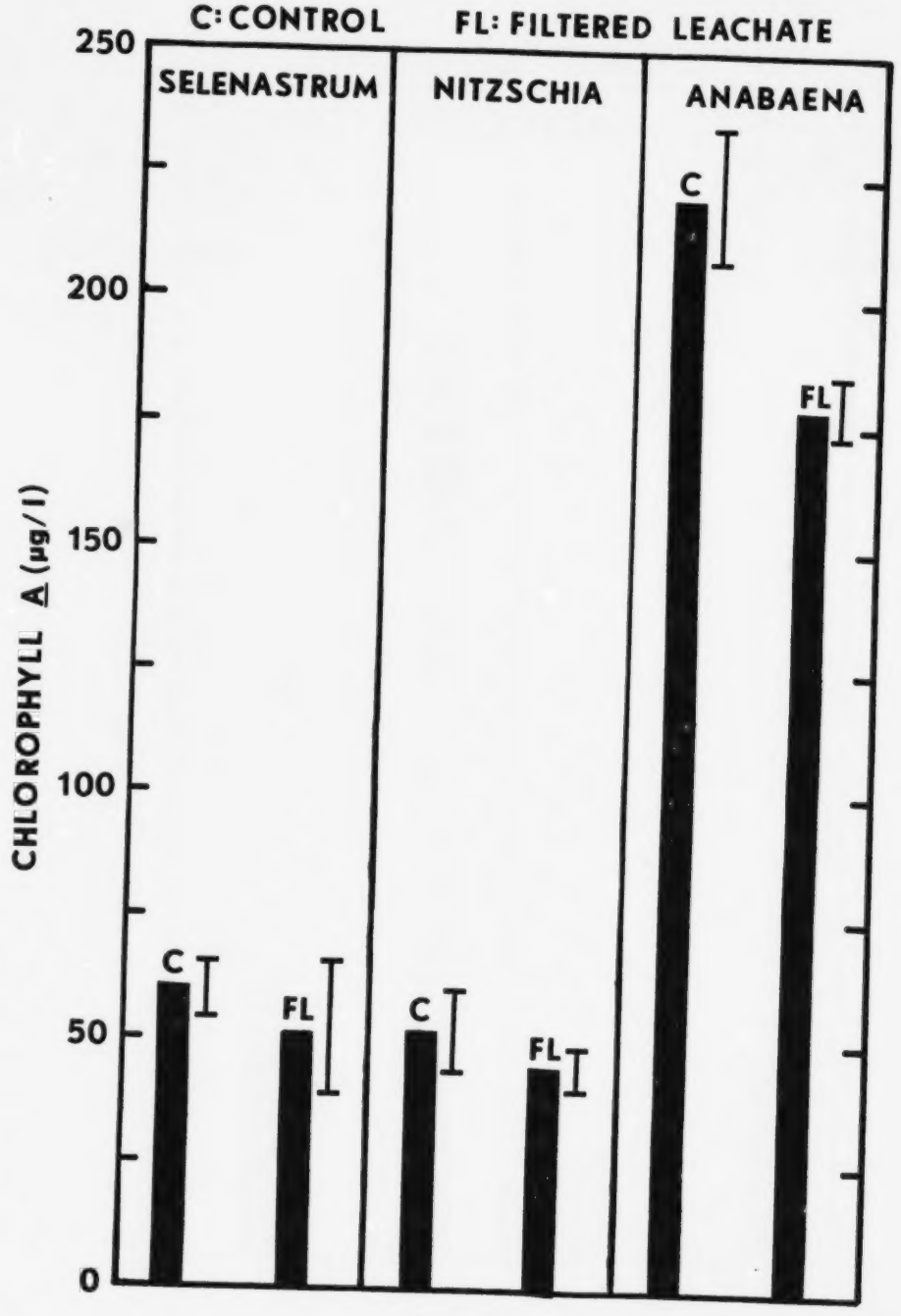

Figure 16. Effects of 20% v/v leachate on the growth of Selenastrum capricornutum, Nitzschia palea, and Anabaena flos-aquae in Lake Superior water in batch cultures, day 5. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Leachate conductivity was 103-132 µmhos/cm; turbidity was 3-5 NTU.

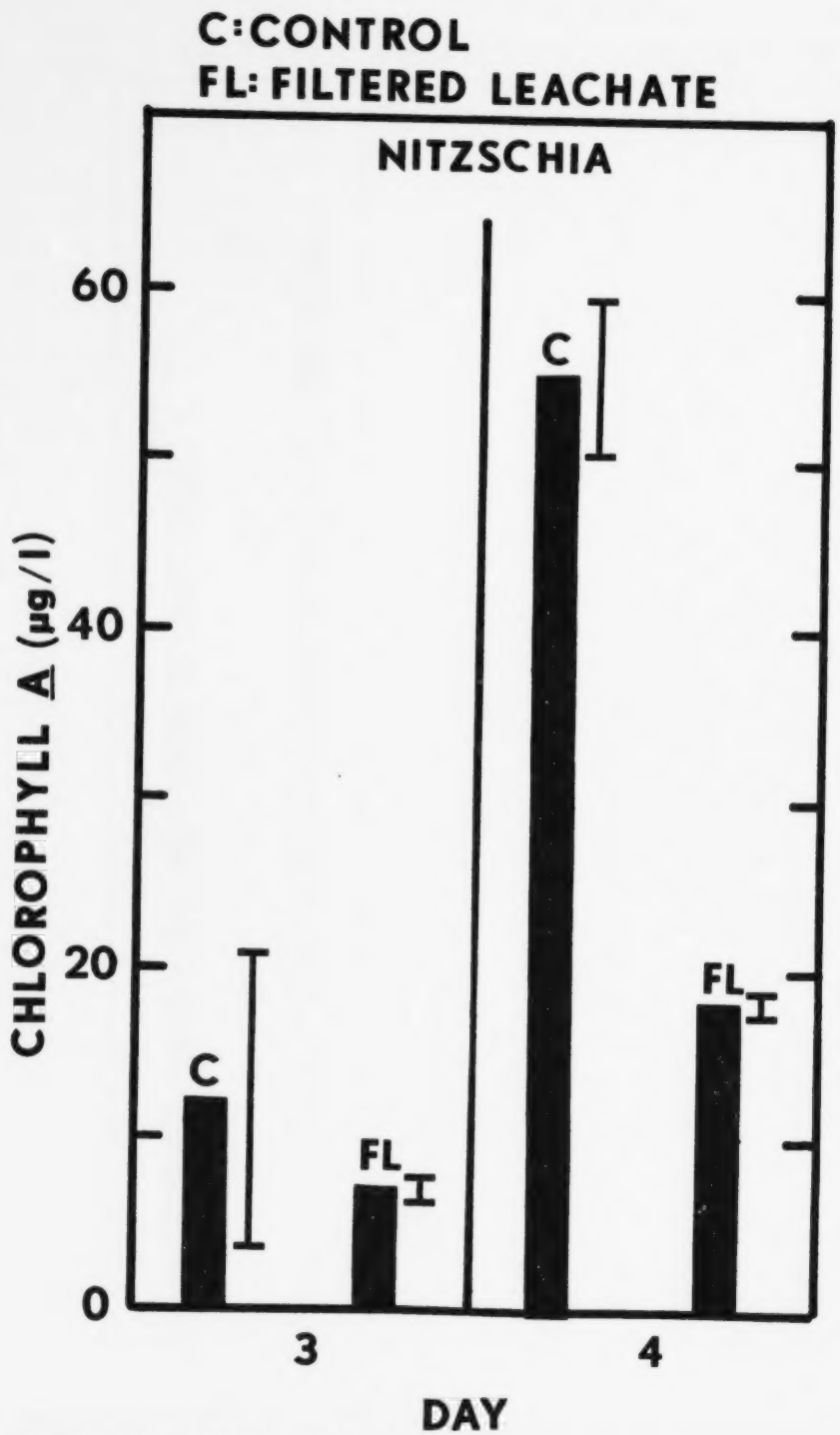

Figure 17. Effects of 20% v/v leachate on the growth of Nitzschia palea in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Leachate conductivity was 71 µmhos/cm; turbidity was 14 NTU.

tissue analysis. Communities were allowed to develop in six streams for 26 days. Following this period, three streams received 12% v/v coal distillate and three received 12% distilled water for 10 days. All streams were enriched with 65 µg/l PO_4-P and 456 µg/l NO_3-N. On day 33 single substrate plates were sampled from each stream, and on day 36 all remaining plates (ten from each stream) were removed and combined into a single composite sample for distillate streams (30 plates) and a single composite sample for control streams (30 plates). After dilution to 2,000 ml, 100 ml was removed from each sample for cell counts and other biomass measurements. The remaining 1,900 ml was centrifuged twice at 15,000 rpm in a continuous-flow centrifuge and the resulting pellets prepared for tissue analysis (see Chemical Analyses).

Stream temperatures averaged 9-12°C during the experiment. No differences in pH or conductivity were observed between treatment and control streams. Chlorophyll a determinations and cell counts suggested somewhat less growth in distillate than control streams (Table 6, Figure 18). Nitzschia spp. were significantly more abundant (95% level) in control than in distillate streams. Tissue analysis showed four peaks in the aliphatic fraction present in the distillate-exposed periphyton which were absent in control periphyton (Figure 19). Also, one of the n-alkanes was present in much higher concentration in the exposed sample. In the aromatic fraction there was a general correspondence of peaks in the distillate and control algal samples, but the quantities of several compounds varied considerably. There was no evident correspondence between dominant peaks in coal distillate and accumulated peaks in exposed periphyton cells. The two periphyton samples were similar in lipid content: 20.5% for exposed algae and 20.3% for control algae.

TABLE 6. CHLOROPHYLL A (µg/cm^2) IN STREAMS TREATED WITH 12% COAL DISTILLATE AND 12% DISTILLED WATER FOR 10 DAYS

Treatment	Day 33 Mean	Day 33 Std. Dev.	Day 36 Mean*
12% Coal Distillate	1.6	0.2	2.2
12% Distilled Water	2.7	0.7	3.1

*Means for duplicate determinations on composite samples.

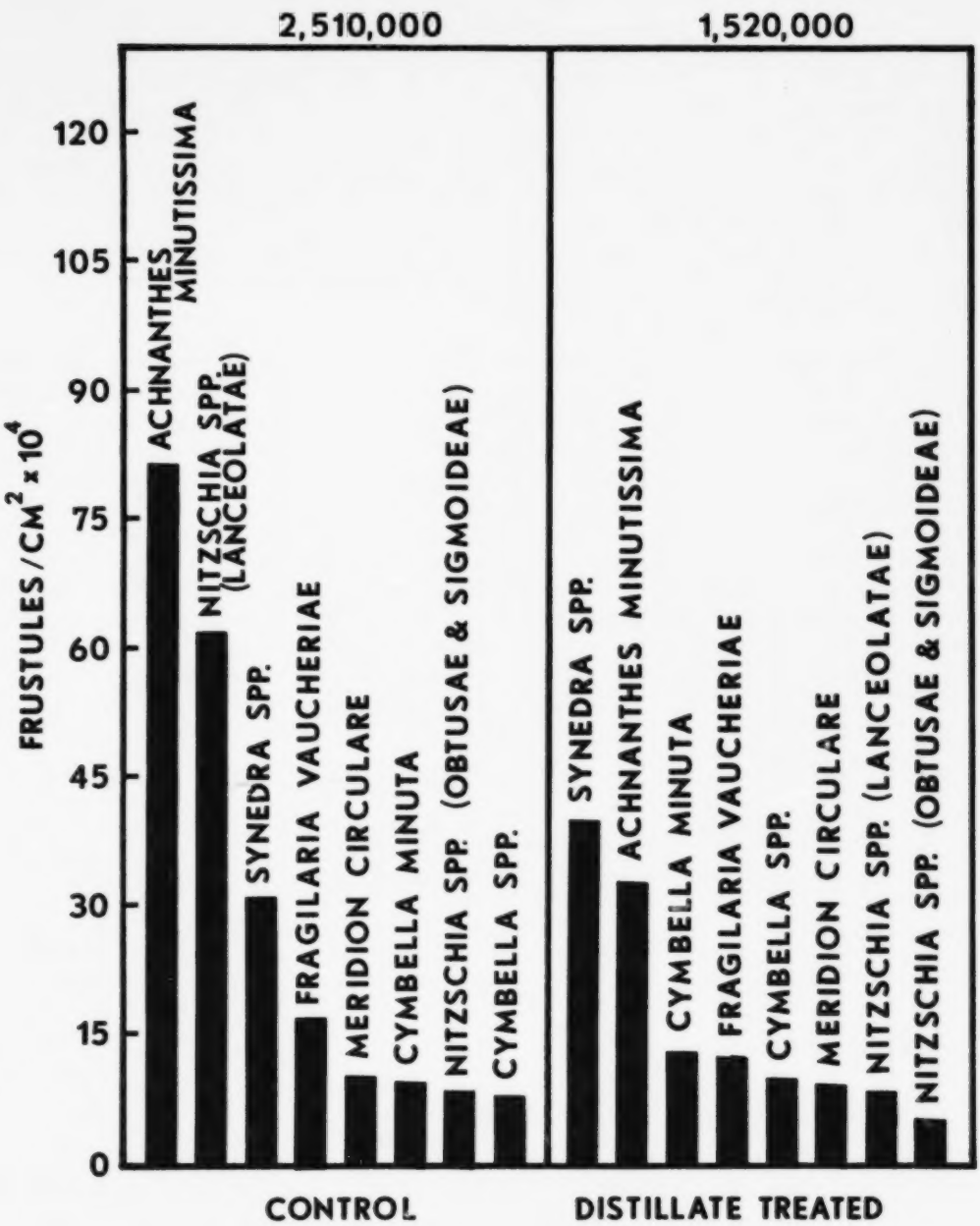

Figure 18. Effects of 12% v/v distillate on species composition and abundance of laboratory stream periphyton, March 1977, day 36. Numbers at top of figure indicate total frustule counts.

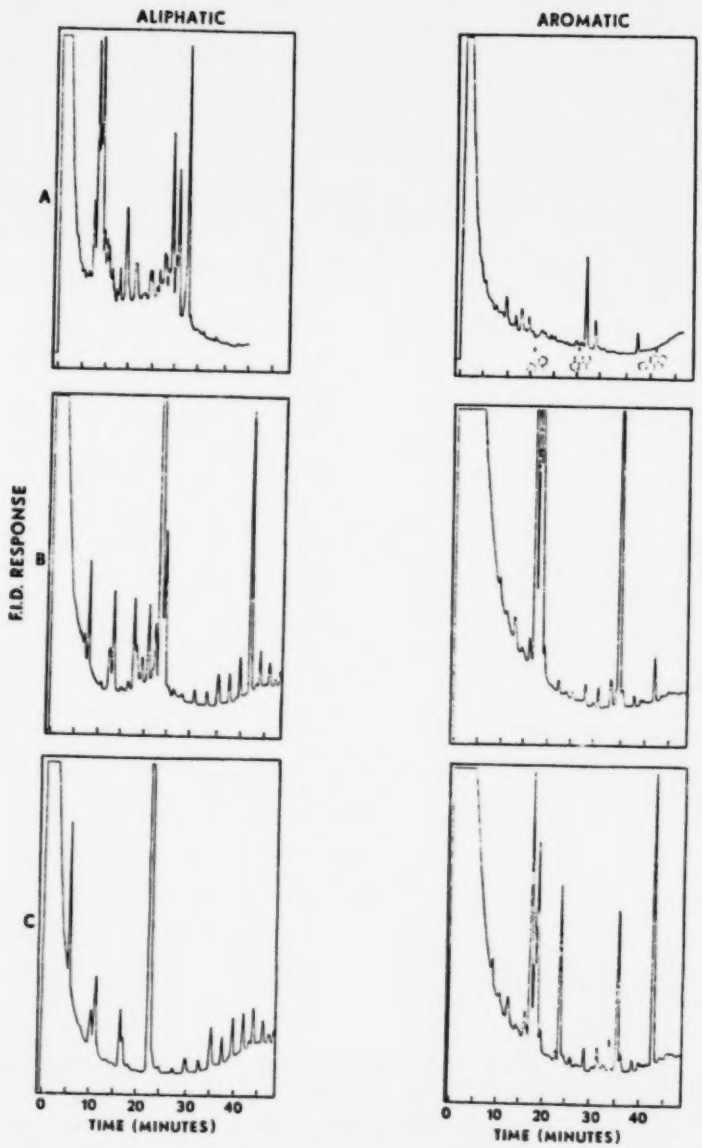

Figure 19. GC analysis of periphyton cells exposed to 12% v/v distillate for 10 days. A- Coal distillate; B- Distillate exposed periphyton; C- Control periphyton.

A similar 10 day dosage with 15% v/v distillate was conducted in May 1978. While chlorophyll *a* and other measures of biomass showed no significant differences between treatment and control streams, *in vivo* fluorescence measurements showed greater percent increases in fluorescence in control streams after the addition of DCMU (Table 7). These results suggest higher photosynthetic rates in control streams (Samuelsson et al., 1978).

TABLE 7. PERCENT INCREASE IN *IN VIVO* FLUORESCENCE AFTER ADDITION OF DCMU IN STREAMS TREATED WITH 15% COAL DISTILLATE AND 15% DISTILLED WATER FOR 10 DAYS*

Treatment	Stream	Substrate Plate	% Increase in Fluorescence
15% Coal Distillate	1	1	55.3
		2	53.3
	2	1	63.0
		2	60.4
	3	1	51.4
		2	62.9
15% Distilled Water	1	1	65.8
		2	70.0
	2	1	70.4
		2	71.4
	3	1	74.5
		2	66.7

*Samples were collected 11 days after the start of the dosage.

Much more dramatic effects on growth were obtained when periphyton were exposed to 15% v/v distillate from the very beginning of a stream bioassay. Such an experiment was conducted in September 1977 when stream temperatures were 14-15°C. UV absorbance values for this distillate were approximately twice those obtained for other distillate samples, indicating higher concentrations of dissolved organics. Chlorophyll *a*, ash-free dry weight, and ATP data indicated severe inhibition of growth in distillate streams (Figure 20). Ratios of dry weight to cellular organic carbon (based on ATP) were generally much higher in distillate than control streams (Table 8), indicating that some non-living organic matter accumulated on the substrates in distillate streams. Diatom frustule counts on day 15 also showed reduced growth in distillate streams (Figure 21). However, the percent abundance of the

dominant species was not very different in distillate and control streams, and most species appeared to be inhibited to an approximately equal degree. An unexpected result of the cell count data was the finding that distillate streams supported as many or more diatom species than control streams (Table 9). However, many of the extremely rare species may have been represented only by dead frustules in both distillate and control streams. Hence, their increased presence in distillate streams was likely due to an increased probability of their being counted when total frustule counts were radically reduced.

TABLE 8. RATIOS OF ASH-FREE DRY WEIGHT TO CELLULAR ORGANIC CARBON IN STREAMS TREATED WITH 15% COAL DISTILLATE AND 15% DISTILLED WATER

Day	Mean for Control Streams	Mean for Distillate Streams
10	35.5	37.4
14	9.9	49.6
15	9.3	51.5
17	5.8	22.2

TABLE 9. NUMBER OF DIATOM SPECIES OBSERVED IN STREAMS TREATED WITH 15% COAL DISTILLATE AND 15% DISTILLED WATER, DAY 15*

Treatment	Stream	Total Frustules Counted	No. Species Observed
15% Coal Distillate	1	7,731	70
	2	7,652	68
	3	4,970	81
15% Distilled Water	1	7,325	55
	2	7,241	58
	3	8,051	52

*The data represent the combined counts from three substrate plates in each stream.

Bottle Tests

A total of 15 small-volume bottle tests of the effects of coal distillate were conducted using test algal species. The control bottles in these tests received volumes of double-distilled, deionized water equivalent to the test volumes of distillate. All flasks were stoppered with foam plugs except where specifically indicated otherwise.

An initial test of the effects of coal distillate on Chlorella sp. was conducted in April 1977. The growth of Chlorella in enriched, membrane filtered Lake Superior water containing 20% v/v coal distillate was monitored using in vivo fluorescence measurements. The calculated chlorophyll a values indicated initial inhibition of growth by distillate (Figure 22).

From September to December 1977 different concentrations of distillate were tested using the alga Selenastrum capricornutum growing in synthetic nutrient medium. Four batch culture tests with 20% v/v distillate showed slight inhibition of growth, determined by haemocytometer counts of algal cells (Figure 23). When these tests were repeated with daily replacement of 50% of the culture volume, the effects were more pronounced (Figure 24).

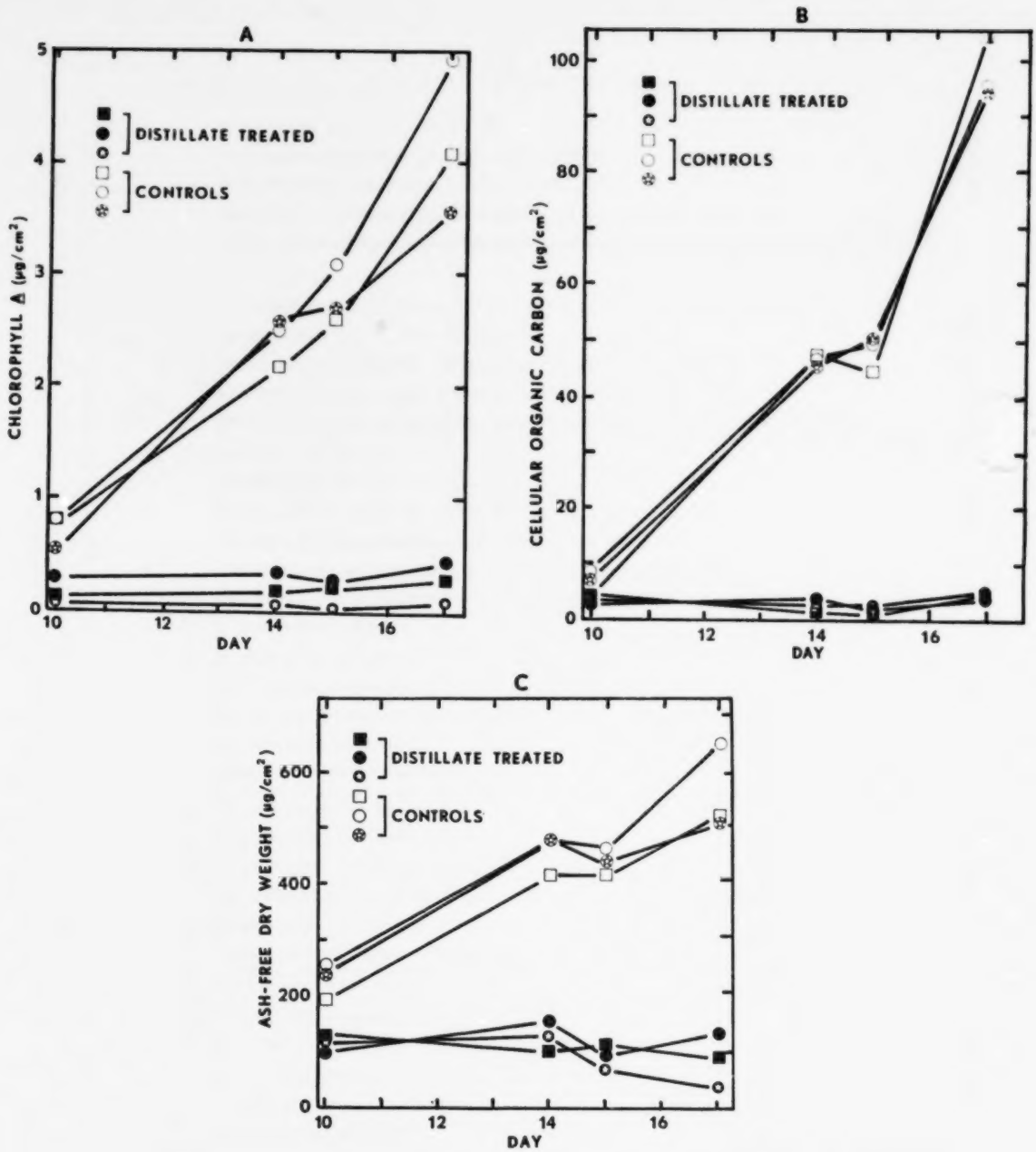

Figure 20. Effects of 15% v/v distillate on laboratory stream periphyton, September 1977. A- Chlorophyll a; B- Cellular organic carbon calculated from ATP analyses; C- Ash-free dry weight.

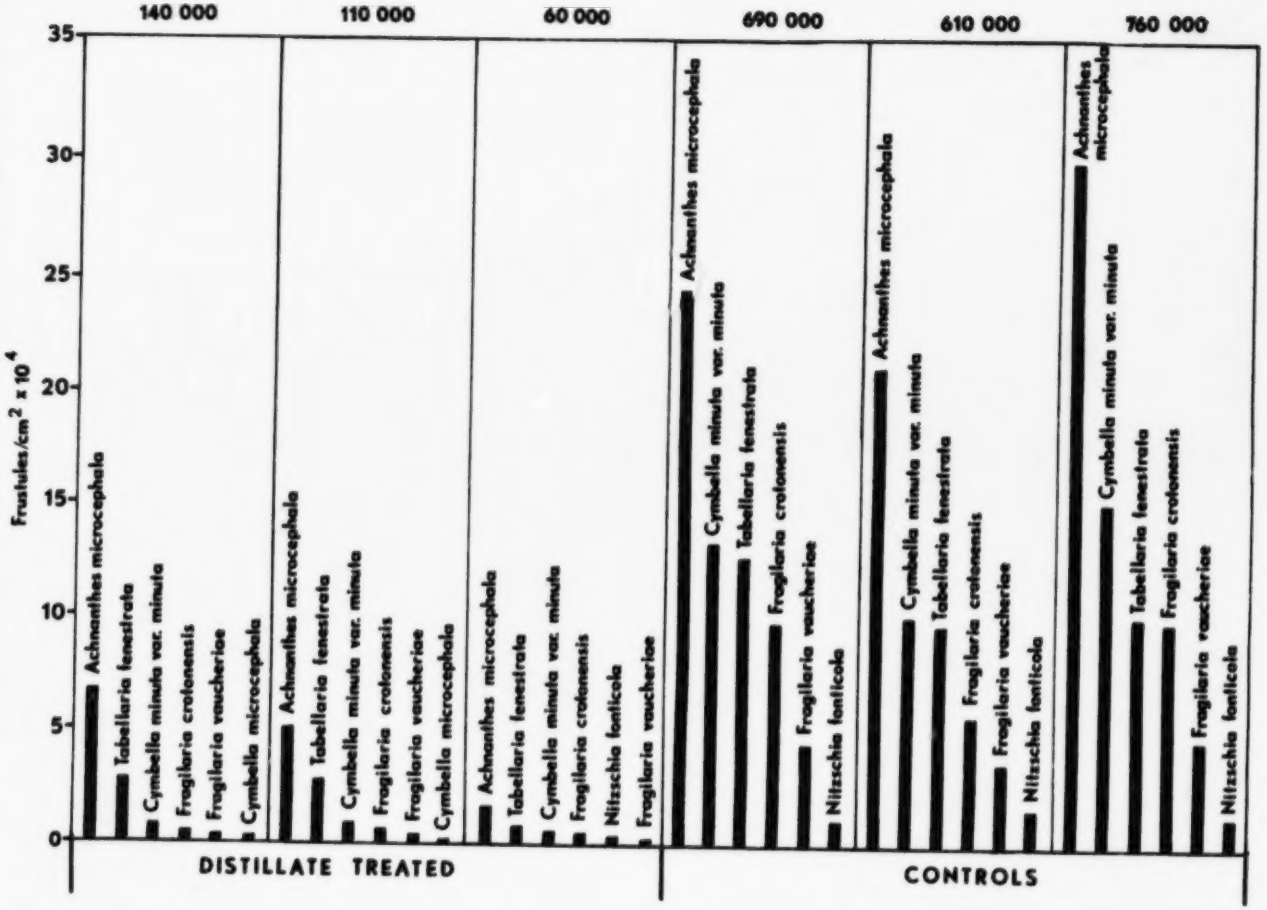

Figure 21. Effects of 15% v/v distillate on species composition and abundance of laboratory stream periphyton, September 1977, day 15. Numbers at top of figure indicate total frustule counts.

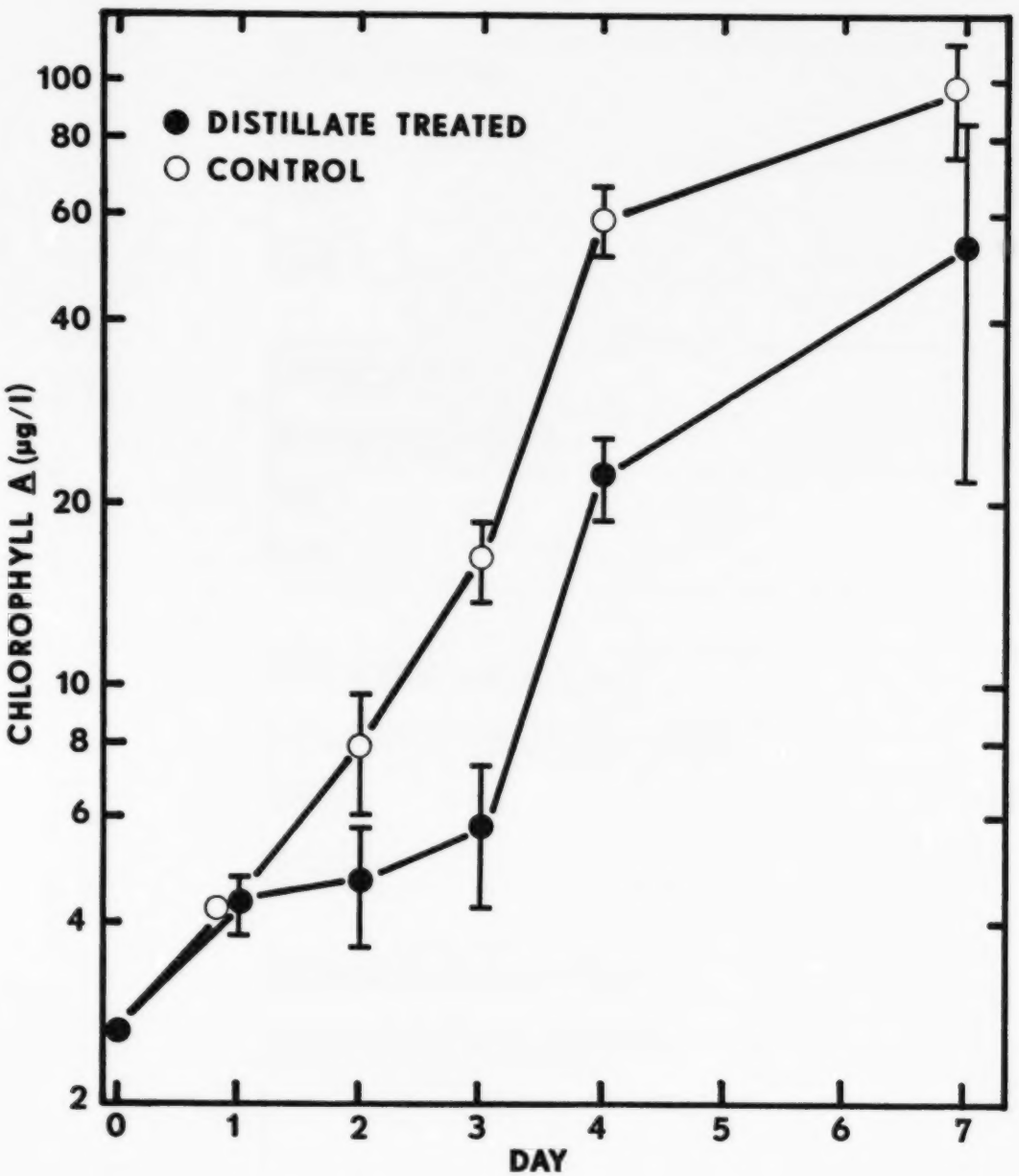

Figure 22. Effects of 20% v/v distillate on the growth of Chlorella sp. in Lake Superior water in batch cultures. Each treatment had 6 replicate flasks. Error bars indicate one standard deviation.

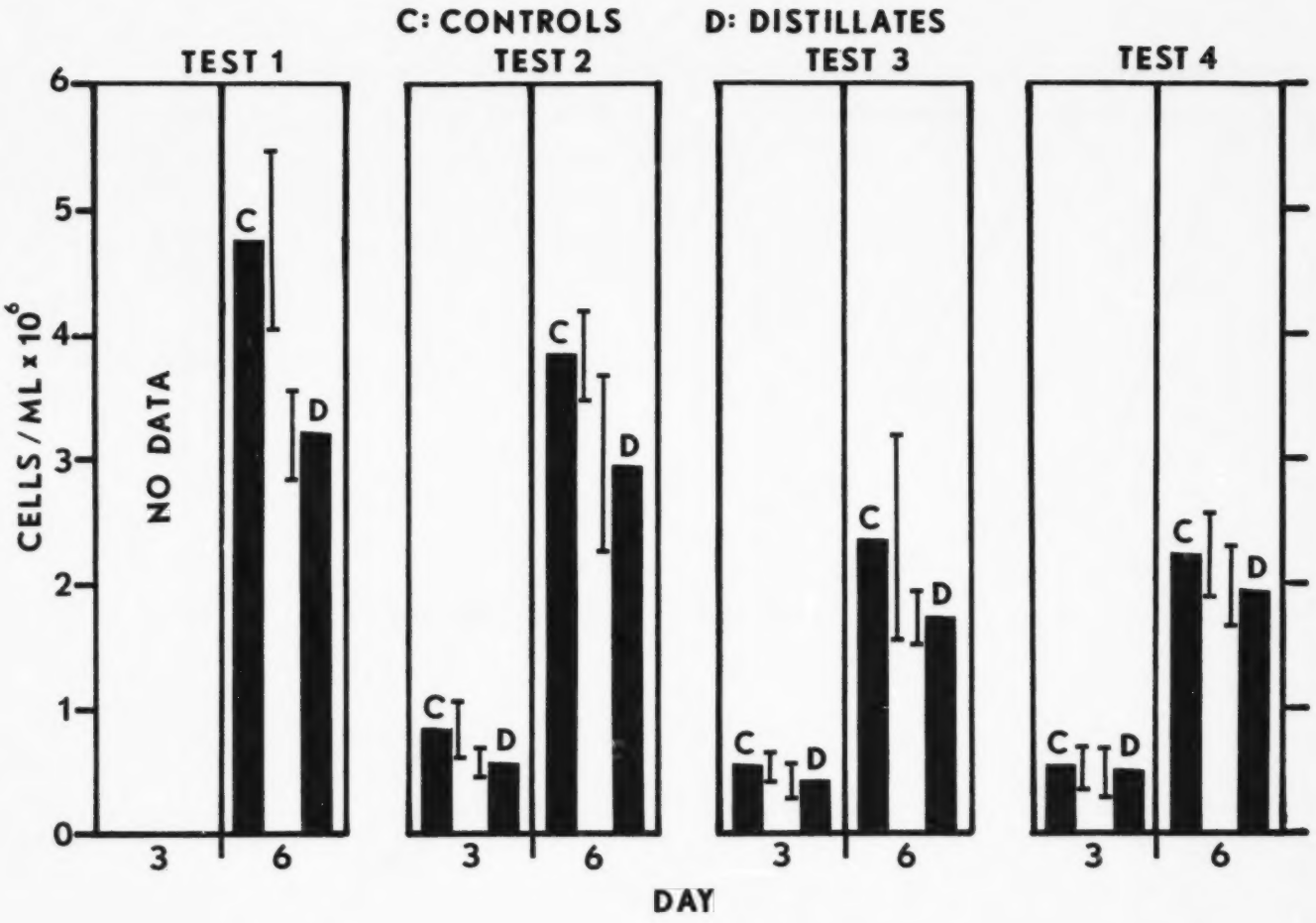

Figure 23. Effects of 20% v/v distillate on the growth of Selenastrum capricornutum in synthetic nutrient medium in batch cultures. Each treatment had 6 replicate flasks. Error bars indicate one standard deviation.

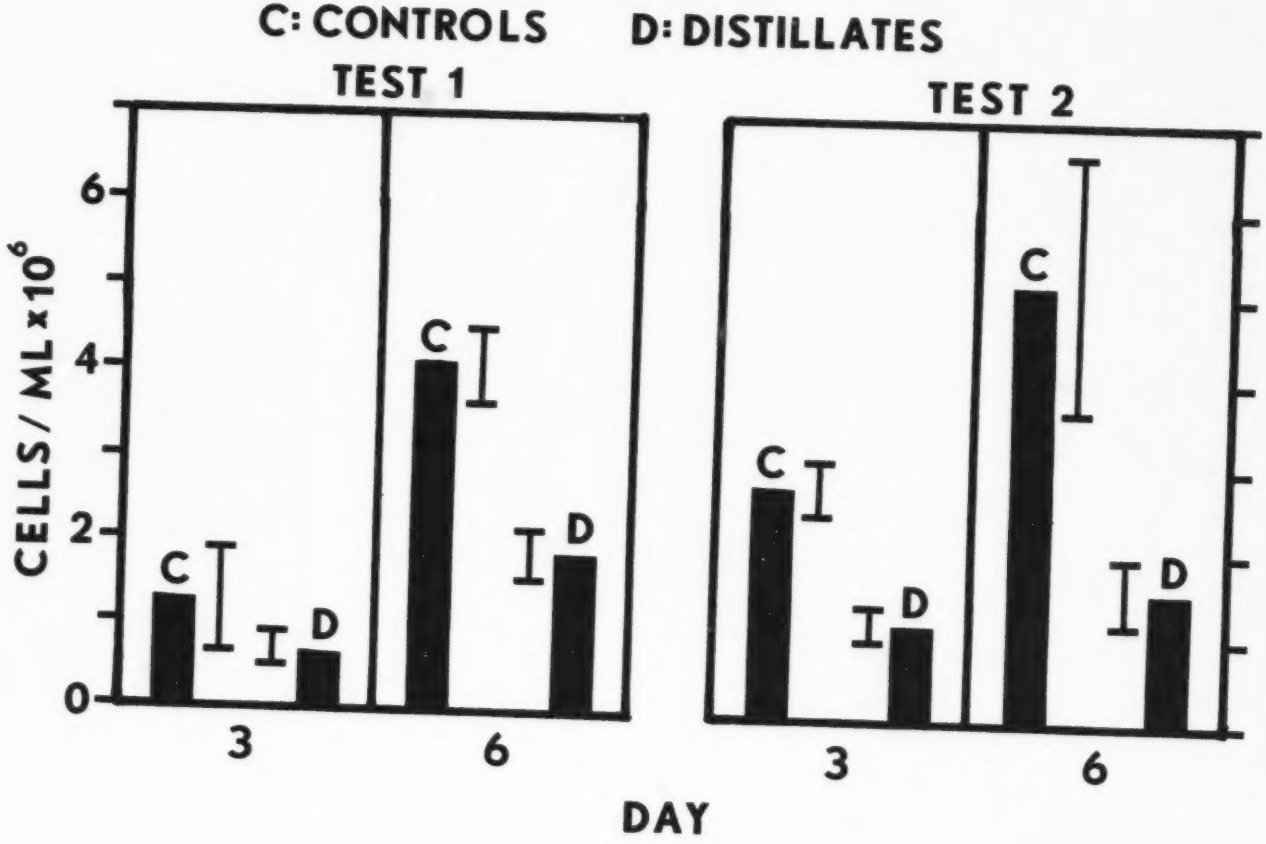

Figure 24. Effects of 20% v/v distillate on the growth of Selenastrum capricornutum in synthetic nutrient medium in 50%/day replacement cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation.

Tests of lower concentrations of distillate with 50%/day replacement cultures were inconsistent. In September two tests were performed with concentrations of 2%, 5%, and 10% v/v distillate. Light intensity was 2,200 lux, and growth was measured by haemocytometer cell counts. In the first test fresh distillate was used, and all concentrations were toxic (Figure 25, Test 1). Distillate used in the second test was stored under refrigeration for one week prior to the test. Concentrations of 5% and 10% v/v again appeared toxic, but the 2% treatment showed no effect (Figure 25, Test 2). The experiments with 2% and 5% v/v distillate were repeated in December using distillate prepared from the same coal sample used in September. Light intensity was 3,700 lux, and growth was measured by cell counts and *in vivo* fluorescence. No inhibitory effects were observed at either concentration, and 2% v/v distillate may have been slightly stimulatory (Figure 26).

Five additional batch tests were conducted in filtered Lake Superior water enriched with 200 µg/l PO_4-P. In these tests chlorophyll *a* was determined by *in vivo* fluorescence measurements after the addition of DCMU, and the percent increase in fluorescence after the addition of DCMU was used as an indicator of photosynthetic capacity. Two tests were conducted with *Selenastrum capricornutum*, two with *Nitzschia palea*, and one with *Anabaena flos-aquae*. In each case the effects of bubbled and unbubbled 20% v/v distillate were examined. In addition, some flasks were stoppered with foam plugs, while others were sealed tightly with neoprene stoppers to prevent the escape of volatile organic compounds. Bubbled distillate was prepared by bubbling distillate samples with filtered air through an airstone for 30-60 minutes. UV spectrophotometric measurements of coal distillate were made in 1-cm far UV quartz cells.

Chlorophyll determinations in neoprene stoppered flasks indicated that the growth of *Selenastrum* was inhibited by unbubbled distillate but may have been stimulated slightly by bubbled distillate (Figures 27A and 27B). In addition, increases in fluorescence after the addition of DCMU were smaller in unbubbled distillate than in control cultures, indicating a reduction in photosynthesis in these flasks. In contrast, unbubbled distillate had little effect in foam stoppered flasks (Figure 27A).

Similar trends were observed using Nitzschia as the test alga (Figures 28A and 28B). *Anabaena*, however, showed no inhibition with unbubbled distillate in neoprene stoppered flasks and may even have been stimulated by this treatment (Figure 29). Unbubbled distillate was, however, inhibitory in foam stoppered flasks. Bubbled distillate had no effect on *Anabaena*.

Considerable increases in pH were occasionally evident in neoprene stoppered bottles, indicating carbon limitation. The magnitude of these increases depended upon the test species and the total yield of algal cells. The highest pH values were recorded in the test with *Anabaena*. Final pH's in this test (day 5) were 8.6-8.7 in foam stoppered flasks and 10.7-10.8 in neoprene stoppered flasks.

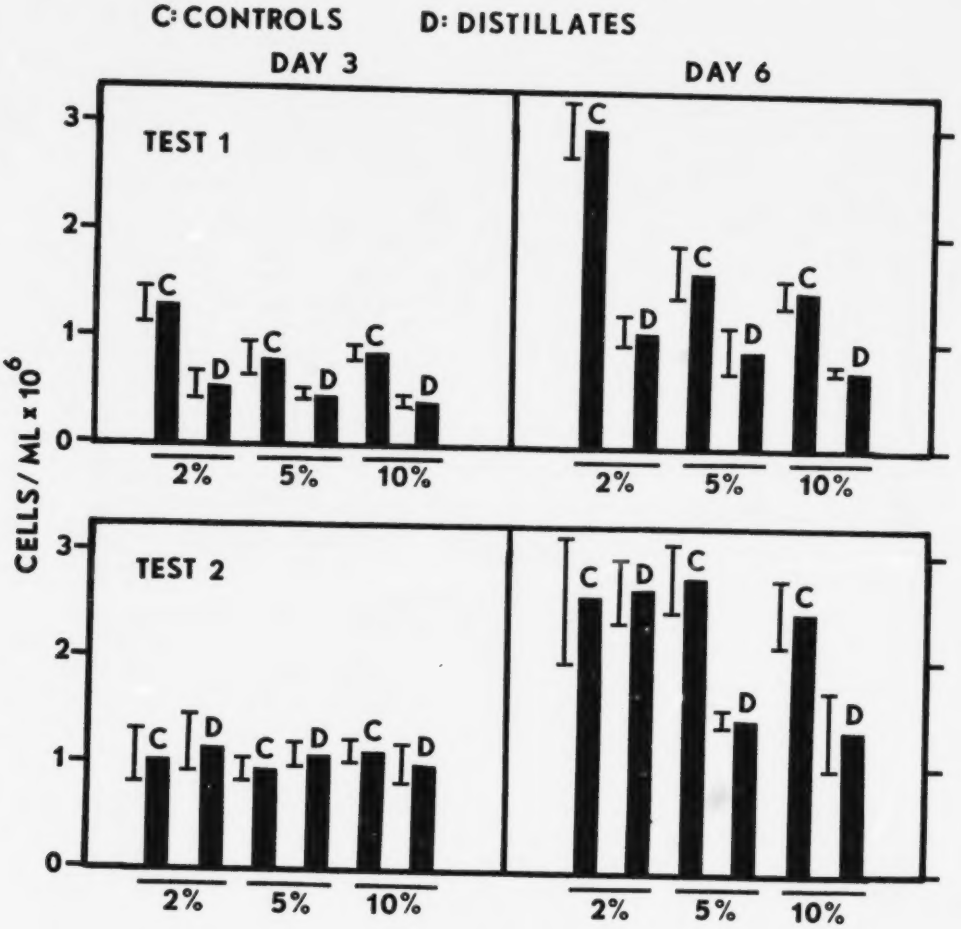

Figure 25. Effects of 2%, 5%, and 10% v/v distillate on the growth of Selenastrum capricornutum in synthetic nutrient medium in 50%/day replacement cultures. Each treatment had 5 replicate flasks. Error bars indicate one standard deviation. Fresh distillate was used in Test 1. Distillate used in Test 2 had been stored under refrigeration for one week.

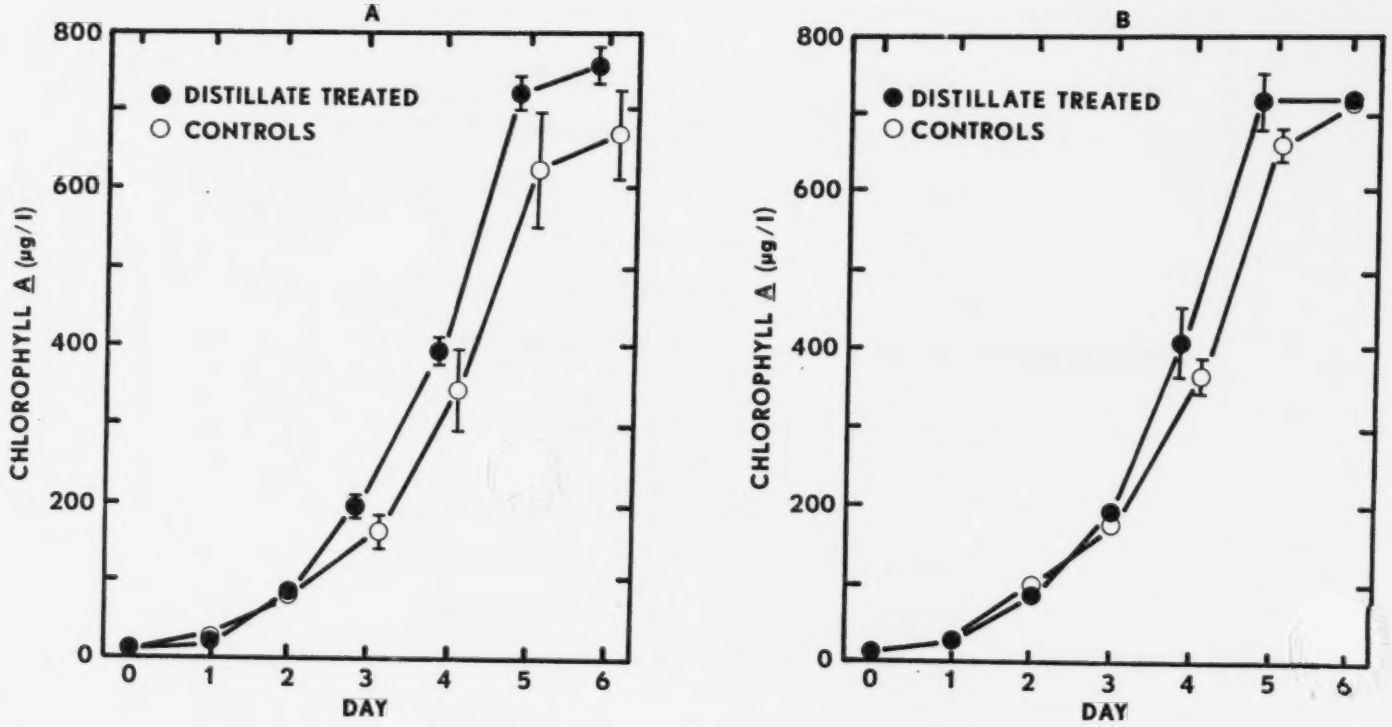

Figure 26. Effects of 2% and 5% v/v distillate on the growth of Selenastrum capricornutum in synthetic nutrient medium in 50%/day replacement cultures. Each treatment had 4 replicate flasks. Error bars indicate one standard deviation. A- 2% v/v distillate; B- 5% v/v distillate.

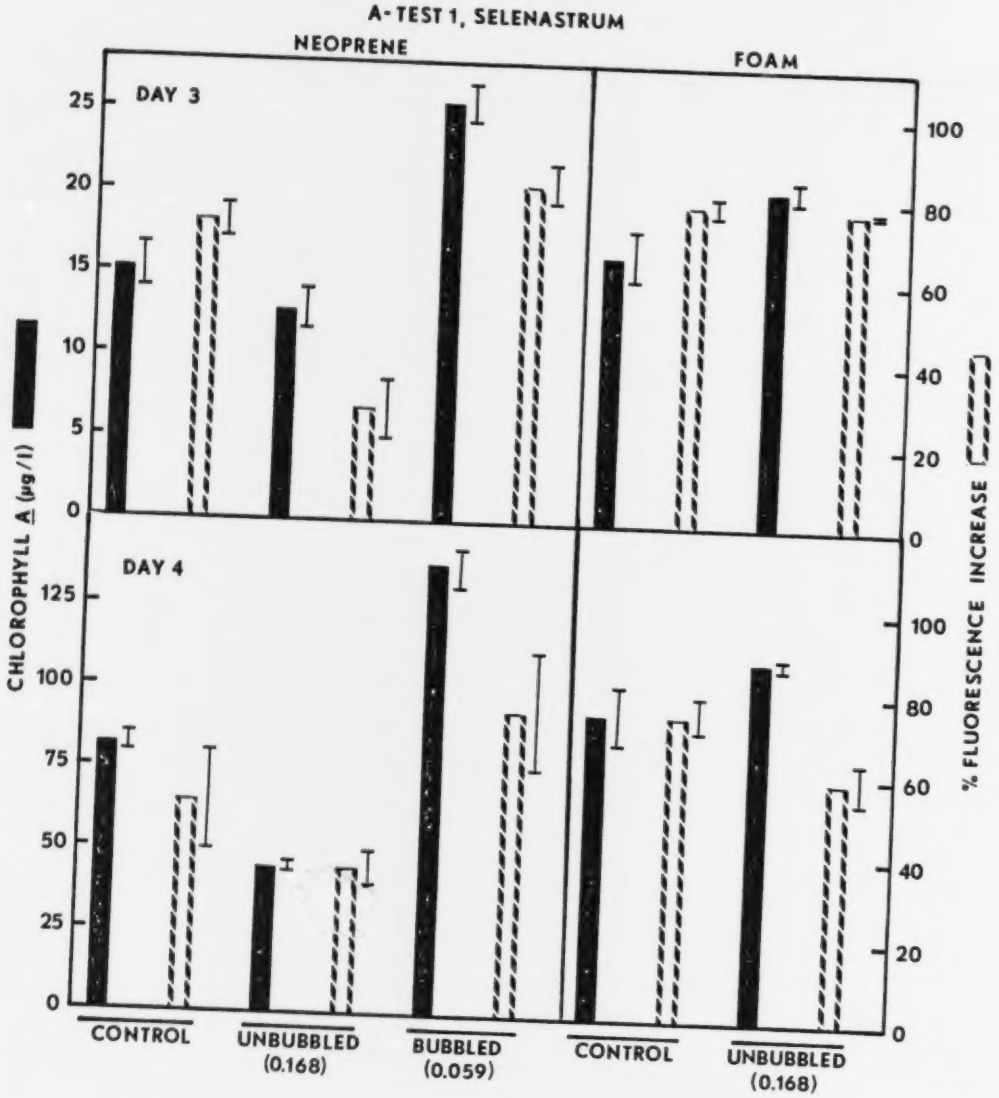

Figure 27A. Effects of 20% v/v unbubbled and bubbled distillate on the growth of Selenastrum capricornutum in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Numbers in parentheses indicate absorbance values of distillate in 1-cm cells at 254 nm. Test 1 employed both neoprene and foam stoppered flasks.

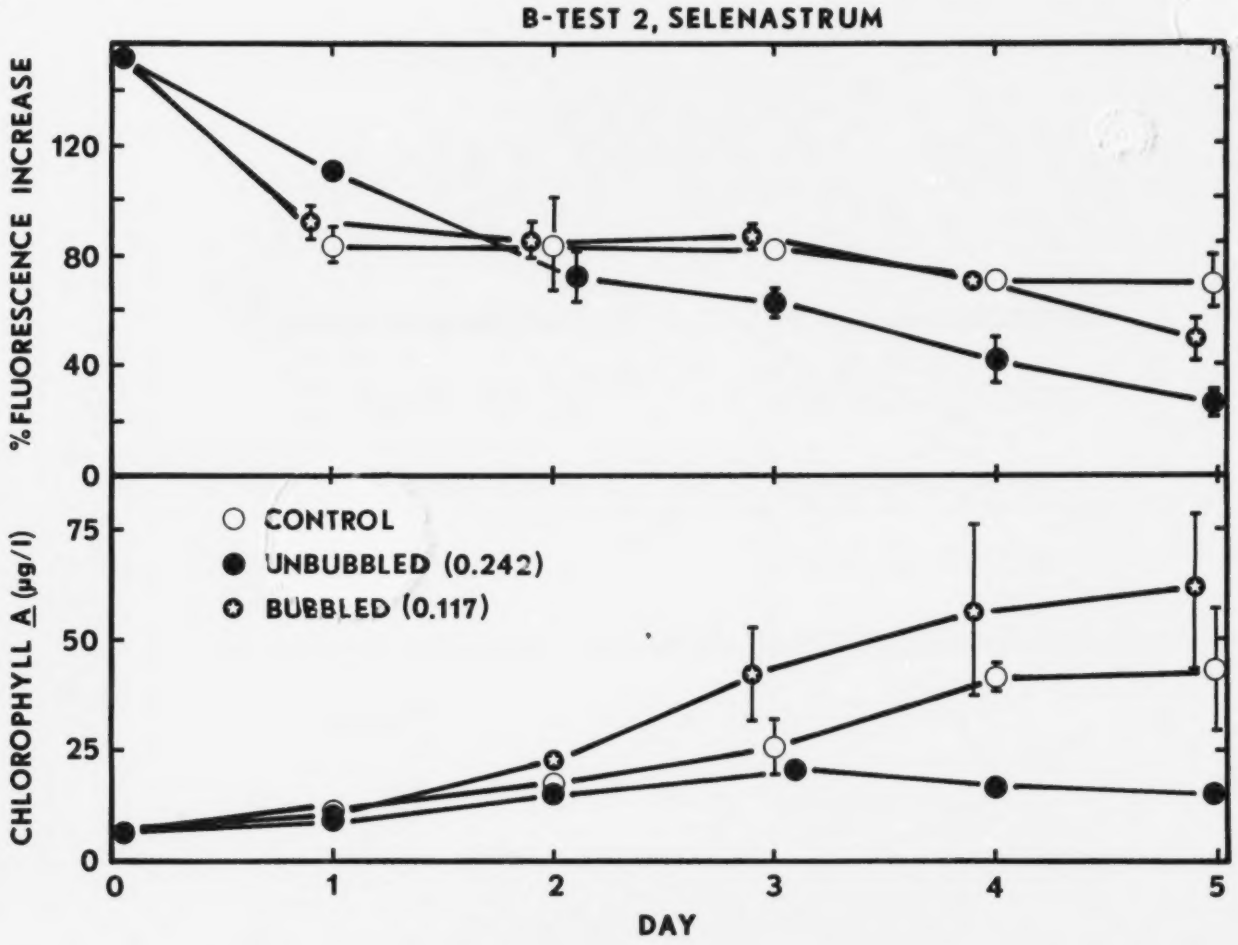

Figure 27B. Effects of 20% v/v unbubbled and bubbled distillate on the growth of Selenastrum capricornutum in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. No error bars are shown where the bar is smaller than the symbol. Numbers in parentheses indicate absorbance values of distillate in 1-cm cells at 254 nm. Test 2 employed neoprene stoppered flasks only.

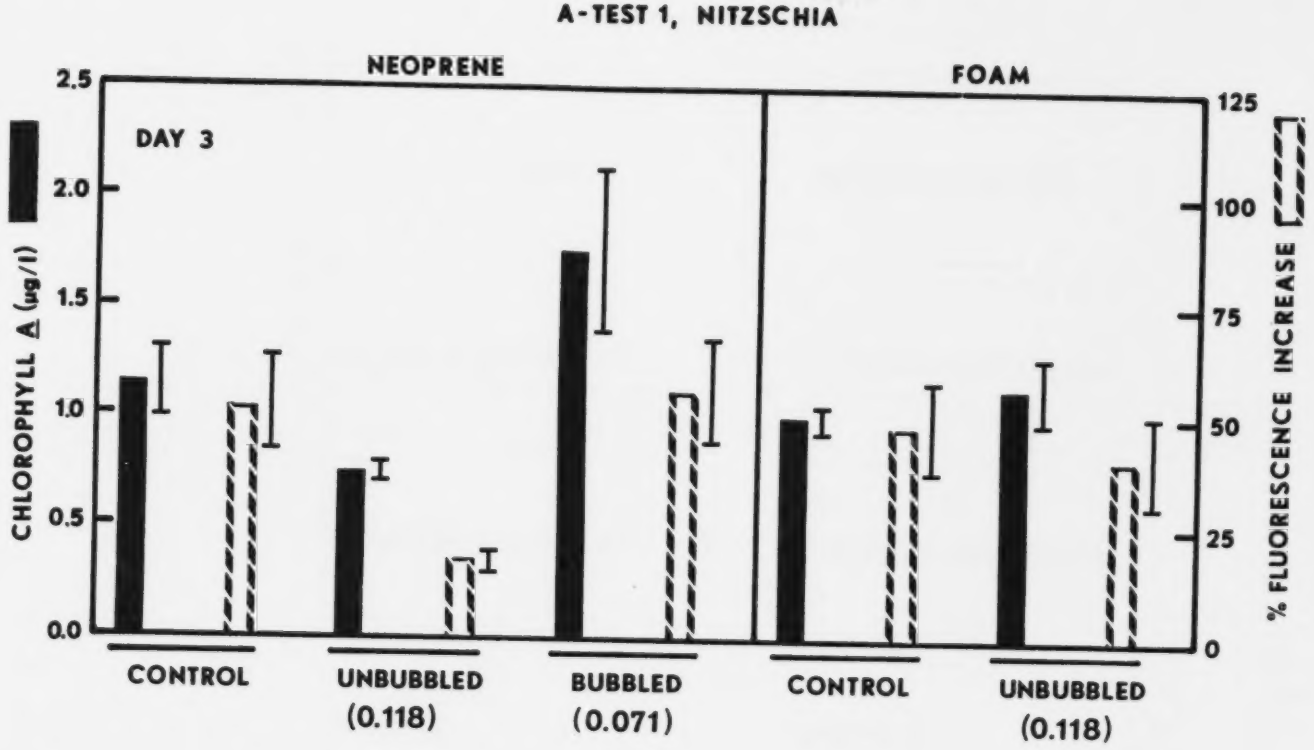

Figure 28A. Effects of 20% v/v unbubbled and bubbled distillate on the growth of Nitzschia palea in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Numbers in parentheses indicate absorbance values of distillate in 1-cm cells at 254 nm. Both foam and neoprene stoppered flasks were employed.

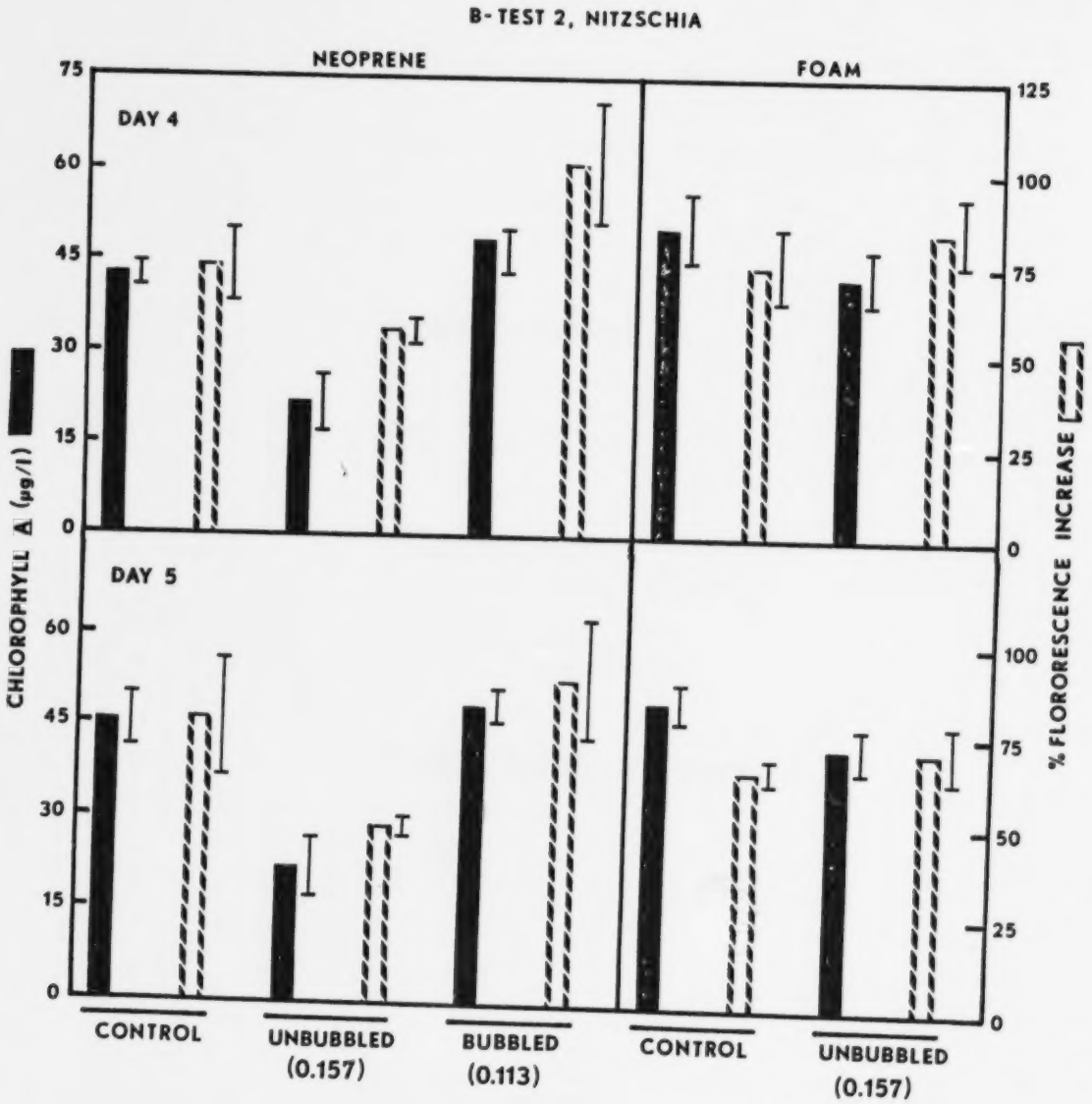

Figure 28B. Effects of 20% v/v unbubbled and bubbled distillate on the growth of Nitzschia palea in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Numbers in parentheses indicate absorbance values of distillate in 1-cm cells at 254 nm. Both foam and neoprene stoppered flasks were employed.

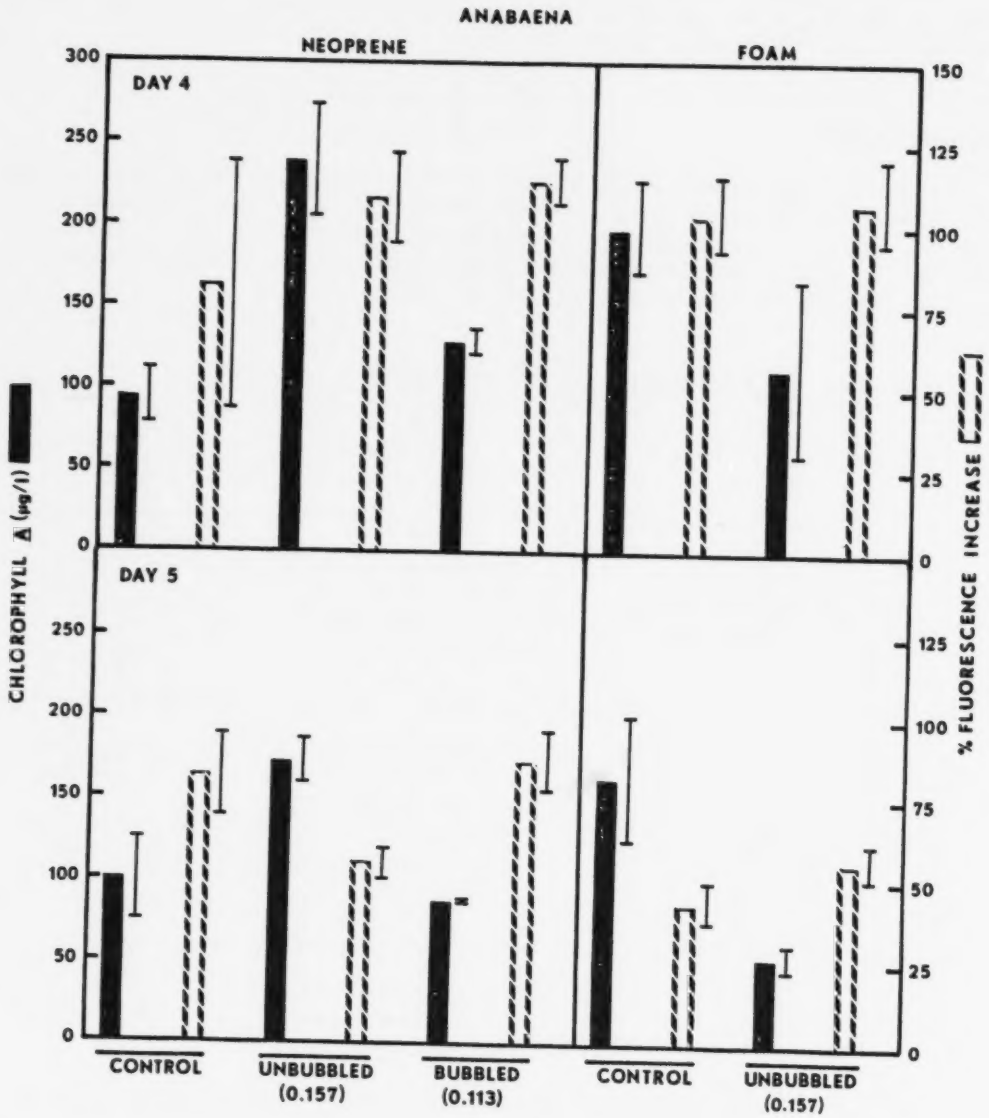

Figure 29. Effects of 20% v/v unbubbled and bubbled distillate on the growth of Anabaena flos-aquae in Lake Superior water in batch cultures. Each treatment had 3 replicate flasks. Error bars indicate one standard deviation. Numbers in parentheses indicate absorbance values of distillates in 1-cm cells at 254 nm. Tests were conducted in both neoprene and foam stoppered flasks.

Bubbling was effective in reducing the concentration of dissolved organics in distillate. Absorbance measurements at 254 nm decreased 28-65% as a result of bubbling (Figures 27-29).

Field Bioassays

Two field bioassays with 1% and 5% v/v coal distillate were conducted on Clearwater Lake during the fall of 1977. The lake was isothermal during these bioassays, with temperatures dropping from 14°C to 6°C. The phytoplankton community was dominated by Dinobryon spp. The experimental design and treatments tested are listed in Table 10.

TABLE 10. EXPERIMENTAL DESIGN FOR FIELD BIOASSAYS

Bioassay	Dates	Treatments	No. of Carboys per Treatment
1	Sept. 28-Oct. 11, 1977	1% distilled water 1% coal distillate 5% distilled water 5% coal distillate	2
2	Oct. 19-Nov. 4, 1977	5% distilled water 5% coal distillate 5% distilled water, filtered 5% coal distillate, filtered	2
3	June 15-June 24, 1978	20% distilled water 20% coal distillate	2

During the first bioassay no toxic effects of 1% or 5% v/v distillate were observed. However, on days 5 and 7 chlorophyll a levels were somewhat higher in bottles treated with 5% distillate than in control bottles (Figure 30). In addition, bacteria populations on day 13 were generally higher in distillate carboys than in control carboys and higher in control carboys than in lake water (Table 11). Nitrate and phosphate concentrations, pH, and other chemical parameters were similar in treatment and control carboys.

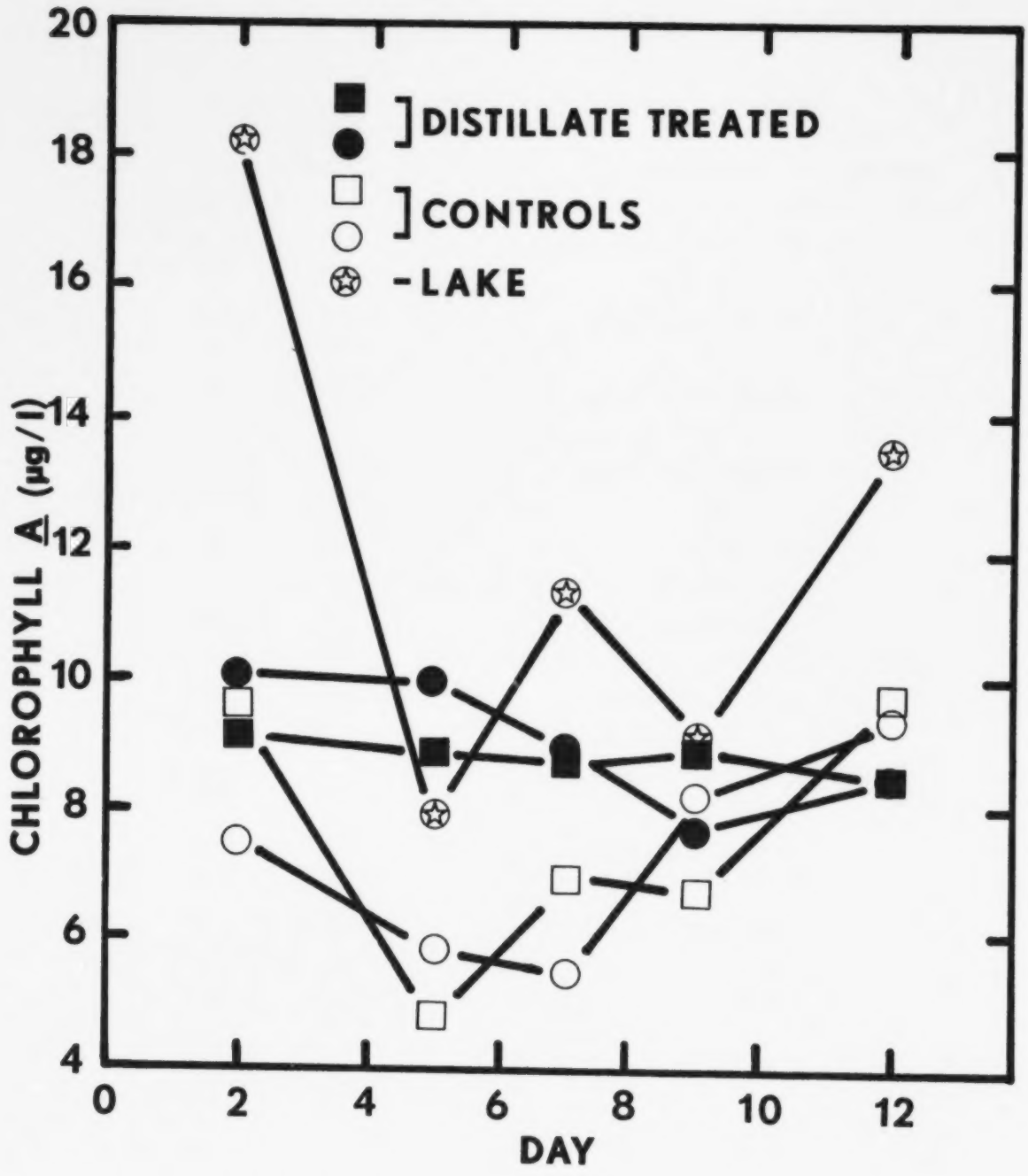

Figure 30. Effects of 5% v/v distillate on Clearwater Lake phytoplankton, September 1977.

TABLE 11. BACTERIA PLATE COUNTS, FIELD BIOASSAYS

Bioassay	Treatment	Carboy	Colonies / ml		Avg.
1 (Day 13)	1% distilled water	1	380	400	
		2	640	700	530
	1% coal distillate	1	1,500	1,840	
		2	900	-	1,413
	5% distilled water	1	960	740	
		2	780	600	770
	5% coal distillate	1	1,560	1,580	
		2	1,500	980	1,405
	Lake water	-	52	107	80
2 (Day 14)	5% distilled water	1	520	700	
		2	1,820	1,580	1,155
	5% coal distillate	1	3,120	3,100	
		2	TNC*	TNC*	-
	5% distilled water, filtered	1	2,700	2,540	
		2	1,280	1,660	2,045
	5% coal distillate, filtered	1	3,580	2,880	
		2	3,660	3,260	3,345
	Lake water	-	194	248	221
3 (Day 9)	20% distilled water	1	3,800	3,800	
		2	4,700	4,800	4,275
	20% coal distillate	1	61,700	65,000	
		2	58,900	37,900	55,875
	Lake water	-	130	104	117

*TNC = Too numerous to count.

A second field bioassay was conducted to verify these results and to determine whether higher chlorophyll levels in distillate carboys were a result of toxic effects of distillate on zooplankters. To test this possibility, zooplankters were experimentally removed from one set of carboys (Table 10) by filtering lake water through an 80-μ plankton net. The net was fitted over the sampling tube prior to pumping lake water into the carboys both at the time of the initial filling and during the daily replacement procedure. Coal distillate used in this experiment had a pH of 4.8 and a conductivity of 25 μmhos/cm. Chlorophyll *a* analyses indicated consistently lower pigment concentrations in control than in treatment carboys on days 5, 7, and 9 (Figure 31). That this effect was observed even in filtered carboys (Figure 31B) suggests that reduced zooplankton grazing in distillate carboys was not the causative factor. Bacterial counts yielded results similar to those of the first experiment (Table 11), and ATP analyses for unfiltered carboys showed higher biomass levels in distillate carboys (Figure 32). Distillate carboys exhibited somewhat lower pH's (6.4-6.5) than did control carboys and the lake (7.0).

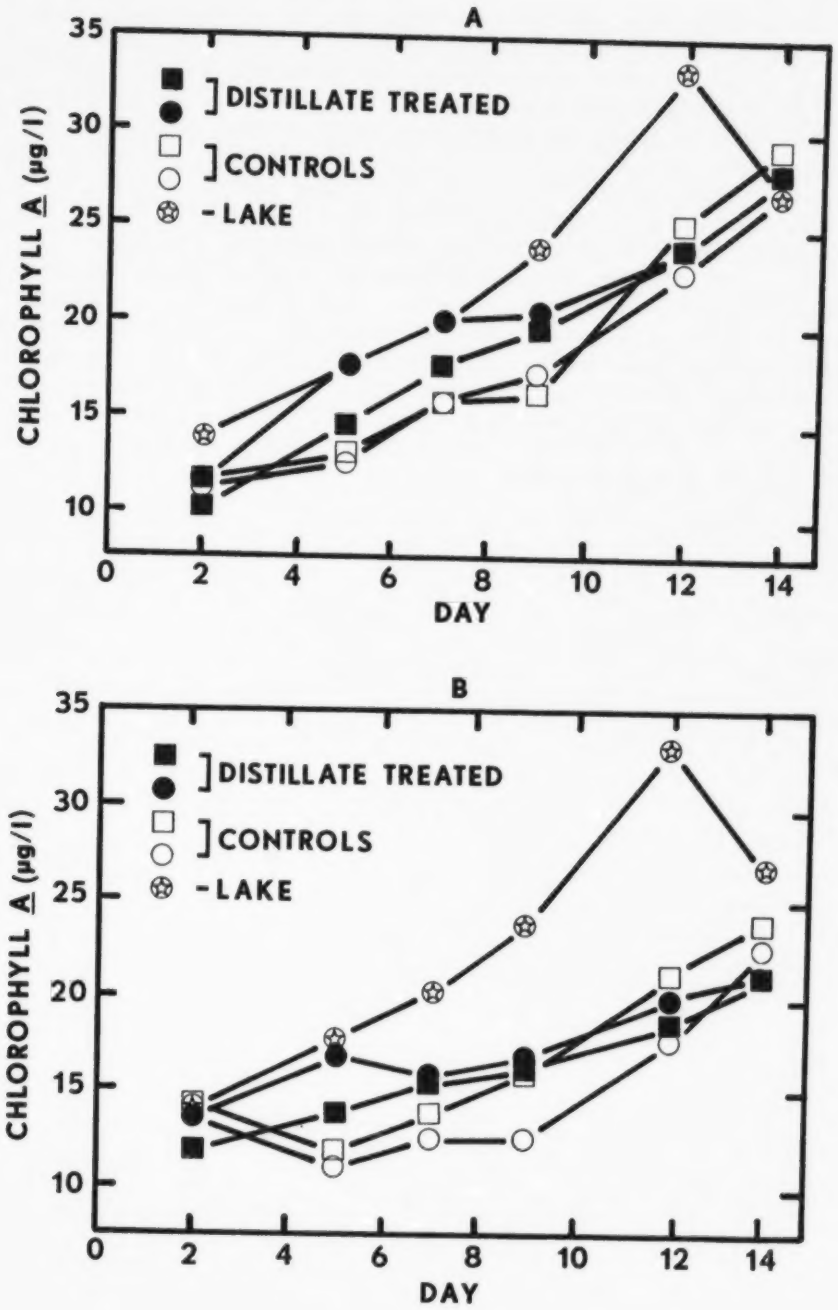

Figure 31. Effects of 5% v/v distillate on Clearwater Lake phytoplankton, October 1977. A- Enclosure water unfiltered; B- Enclosure water filtered through an 80-µ plankton net.

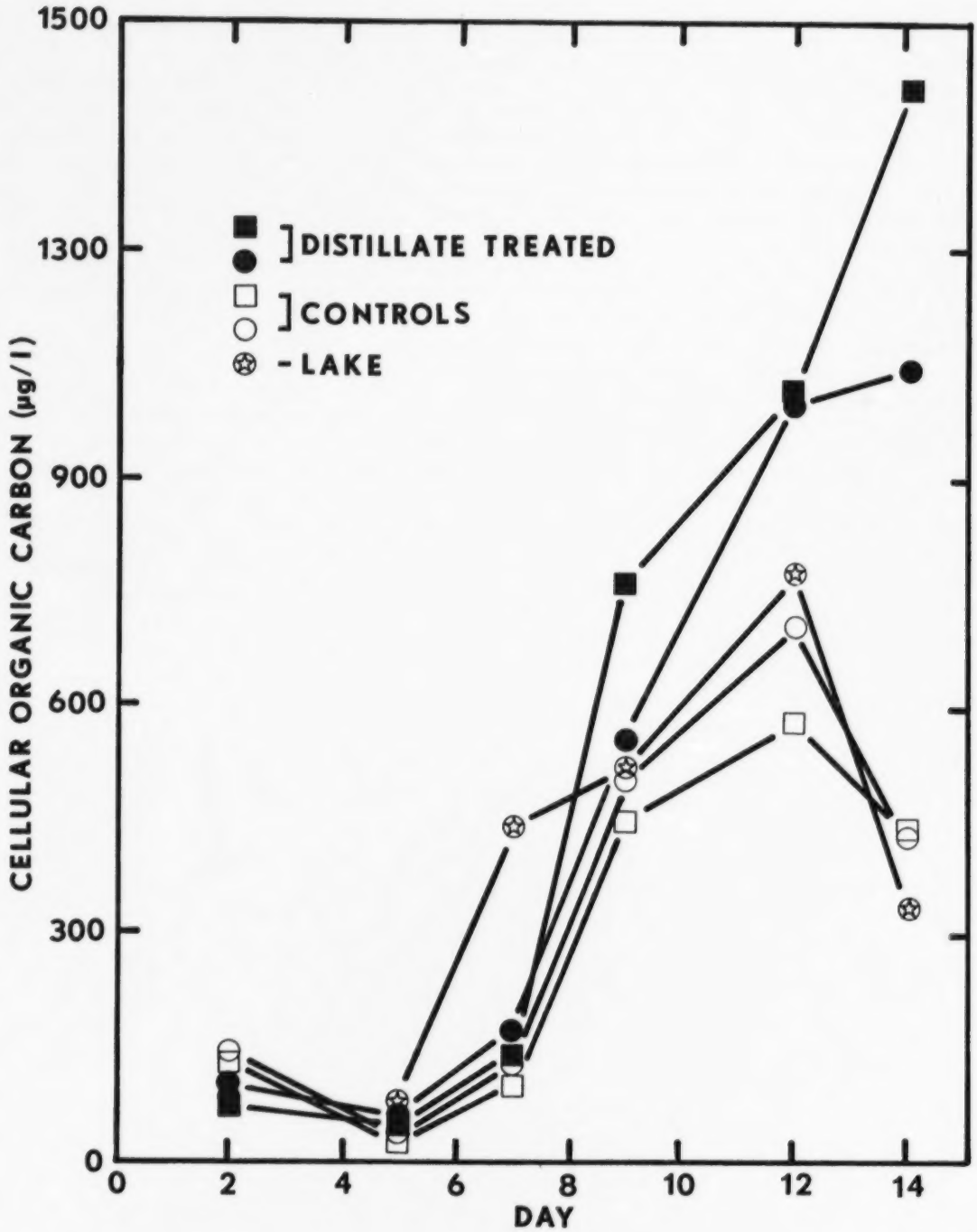

Figure 32. Effects of 5% v/v distillate on Clearwater Lake planktonic biomass, October 1977, unfiltered carboys. Values for cellular organic carbon were calculated from ATP analyses.

A final field bioassay was conducted in June 1978 using 20% v/v distillate (Table 10). The distillate used in this bioassay had a pH of 4.7 and a conductivity of 11 μmhos/cm. UV absorbance of this distillate at 254 nm was only half that of distillate used in the fall 1977 experiments. The results of chlorophyll analyses and bacterial plate counts (Figure 33, Table 11) were similar to those observed in the previous tests, with distillate carboys showing higher chlorophyll concentrations on days 5 and 7 and much higher bacterial counts than control carboys. On day 5 when chlorophyll reached peak values in distillate carboys, photosynthetic capacity also appeared to be somewhat greater in distillate carboys. The percent increases in fluorescence after the addition of DCMU were 152 and 163 in distillate carboys and 70 and 95 in control carboys on this day. In spite of higher chlorophyll and bacteria counts in distillate carboys, ATP analyses indicated lower total biomass in these enclosures (Figure 34). This was true for all sampling days when whole water samples were analyzed (Figure 34A), but only for days 3 and 5 when samples which had been filtered through a 48-μ net were analyzed (Figure 34B). Both ATP and ash-free dry weight analyses indicated that a precipitous decline in biomass occurred in the lake during the course of the experiment. Field and laboratory observations of zooplankton samples collected in 80-μ net indicated that nearly all zooplankters were killed in distillate carboys, while zooplankters appeared in good condition in control carboys. The pH in distillate carboys was significantly lower (avg. = 5.9) than in control carboys (avg. = 6.5) or in lake water (avg. = 6.6).

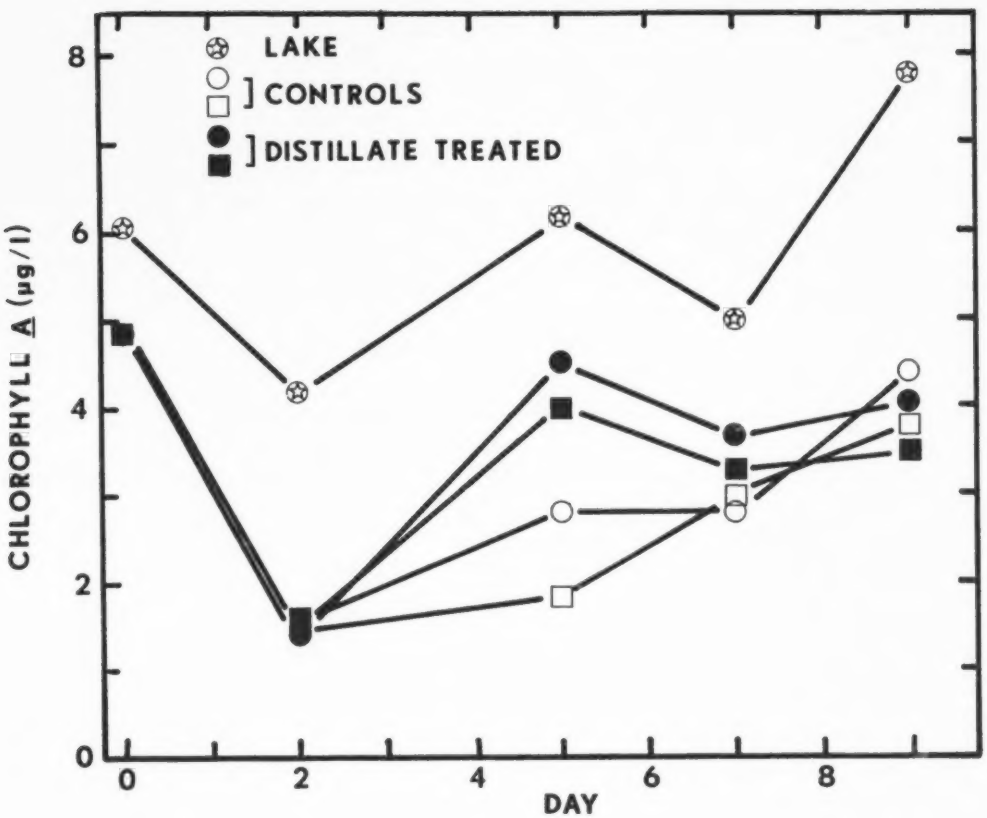

Figure 33. Effects of 20% v/v distillate on Clearwater Lake phytoplankton, June 1978.

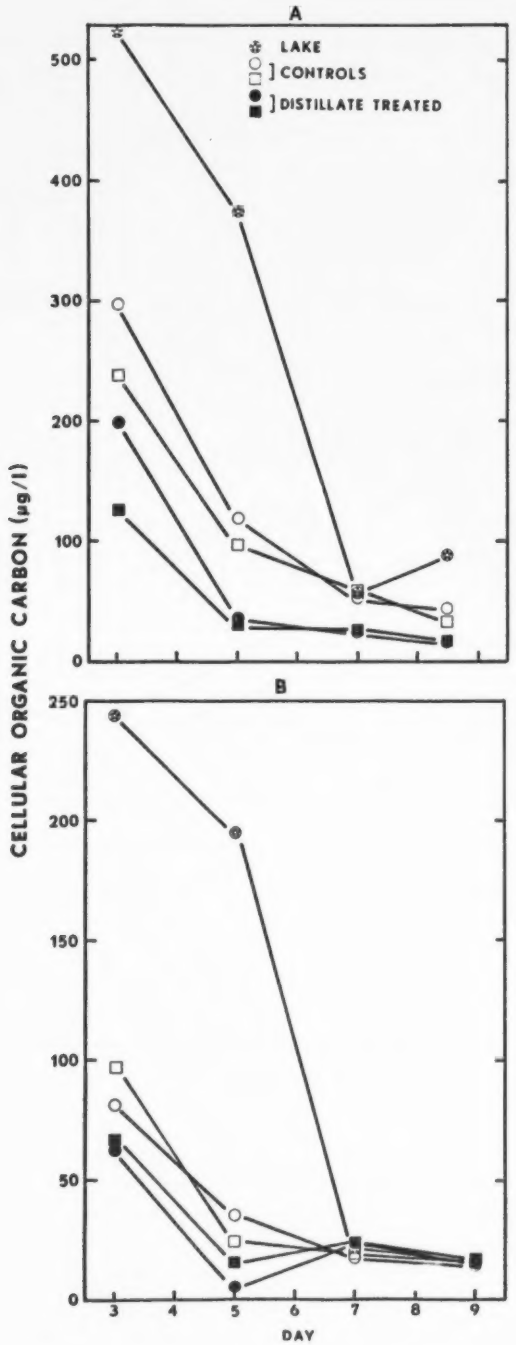

Figure 34. Effects of 20% v/v distillate on Clearwater Lake planktonic biomass, June 1978. Values for cellular organic carbon were calculated from ATP analyses. A- Whole water samples; B- Water samples filtered through a 48-µ net.

SECTION 6

DISCUSSION

Although coal leachates usually stimulated periphyton growth in laboratory streams, filtered leachates inhibited the growth of test algae in bottle tests. At present, this contradiction between the results of stream and bottle bioassays cannot be adequately explained. Important differences between the two bioassay types include algal growth habit, the presence of a multispecific association of bacteria and algae in the stream bioassays, and the use of polyethylene and plexiglass in the laboratory stream structure vs. glass enclosures for the bottle bioassays. However, in tests with copper and nickel, Gerhart and Davis (1978) likewise found that algae in laboratory cultures showed more extreme responses to additions of toxicants than algae in field enclosures, and copper was occasionally toxic in laboratory experiments but stimulatory in the field. This study employed multispecific communities of algae contained in glass enclosures in both field and laboratory. Additional work with coal leachates using in situ lake enclosures would be desirable.

Some speculation is possible concerning the components of leachates which produced these growth responses. Simple leaching of western coals with pure water has been shown to remove only small amounts of metals (Coward et al., 1978), and our own analyses also suggest that metals were unimportant. Organic phosphorus in leachates was initially suspected as a source of phosphorus for algal growth in stream bioassays. However, no evidence in support of this theory was obtained, and in one experiment growth in leachate and control streams was equally slow before the addition of inorganic phosphorus to the lake water. In addition, no evidence of stimulation was observed in bottle tests conducted at low phosphorus concentrations. It seems likely that the different responses of the algae in stream and bottle bioassays occurred in response to the same component of coal leachate. There are many examples of algae responding by both growth enhancement and inhibition to the same material under different conditions (e.g., Niemi, 1972; Kauss et al., 1973; Gordon and Prouse, 1973; Dunstan et al., 1975). Both metals and organic compounds have been observed to produce these dual effects. Such studies clearly suggest that algae may respond to some chemical stresses with a generalized increase in growth.

Non-polar aromatic organic compounds were not important in leachates. Carlson et al. (1978) found concentrations of these compounds in leachates to be similar to background concentrations in Lake Superior. Polar materials were also present in leachates, but their kinds and concentrations were not

determined. Our own analyses indicated that phenolic compounds were present in both leachates and distillates. Since some algae are known to degrade phenols (Ellis, 1977), a stream bioassay was performed using phenol concentrations of 0.35 and 1.1 mg/l. These concentrations are 2-7 times higher than the concentration measured in a sample of coal leachate. No effect on periphyton growth was found. Thus, the effects of dissolved organic compounds in leachates are probably quite small, but a more complete investigation of this topic is needed. For example, it is possible that the phenolic compounds in leachates are substituted forms which are more toxic than the unsubstituted phenol tested in the stream bioassay or that the indigenous periphyton in the laboratory streams were able to utilize organics more efficiently than the bacterial-algal association present in the bottle tests.

Coal particulates in leachates are suspected of causing algal responses in both stream and bottle bioassays. In the streams, particles of less than 1 μm diameter were observed to be consistently and intimately associated with growing periphyton cells. The effects of these particles, perhaps in combination with bacterial activity, are unknown. In bottle tests, the toxicity of membrane filtered leachates was apparently correlated with turbidity and not with conductivity, suggesting an important role for extremely fine (<0.45 μm) coal particles. Tests indicated that the effects of particles in leachates were not simply a result of light attentuation in either stream or bottle bioassays. Adsorption or chelation of trace nutrients by particulate or dissolved organics in coal could conceivably contribute to reduced growth in bottle tests, but it is not clear why similar effects did not occur in stream bioassays, nor is significant adsorption or chelation likely in bottle tests employing the synthetic nutrient medium. Of interest in this regard are studies conducted by Ishio et al. (1972a, b) of the marine alga Porphyra tenera growing on sea bottom mud heavily polluted by wastes discharged by the coal chemical industry. These authors reported that suspended solids in the mud are the cause of a cancerous disease of the alga. In addition, Coward et al. (1978) found that coal particles from western coal may diminish the growth of Lemna minor. Our data similarly suggest, but do not prove, that suspended fine particulates in coal leachates are the likely cause of algal growth stimulation in stream bioassays and of inhibition in bottle bioassays.

All of our leachate bioassays were conducted with centrifuged or filtered leachates. Thus, uncontrolled runoff from coal storage piles could result in concentrations of suspended solids many times higher than those tested in these experiments. Reduction of light penetration and loss of suitable substrates for periphyton growth are additional effects which could become important under these conditions.

The bioassays with coal distillates are perhaps more easily interpreted than those with leachates. Bottle bioassays in which both bubbled and unbubbled distillates were tested showed that volatile organics were almost certainly responsible for the toxic effects of distillates. The majority of the compounds volatilized from coal appear to be alkanes, alkylbenzenes, and alkylnaphthalenes (Carlson et al., 1978). A frequent result was

toxicity at higher concentrations of volatile organics (unbubbled distillates) and growth stimulation at lower concentrations (bubbled distillates). Growth stimulation observed with bubbled distillates could be interpreted as resulting from CO_2 introduced during the bubbling process rather than from low concentrations of volatile organics (Figures 27 and 28), and we recommend nitrogen gas for stripping organics in future work. However, since total growth in foam stoppered control flasks was not significantly greater than in neoprene stoppered control flasks, we believe this alternative interpretation is unlikely. Our experiments also demonstrated species specific differences in algal responses and the need to tightly stopper all bioassay flasks containing volatile organic materials. Neoprene rubber stoppered flasks often showed distillate toxicity while foam stoppered flasks were similar to controls, a finding similar to that of Atkinson et al. (1977). All of these results are supported by the now extensive literature dealing with the effects of fuel oils and low molecular weight hydrocarbons on algae (e.g., Gordon and Prouse, 1973; Kauss et al., 1973; Pulich et al., 1974; Dunstan et al., 1975; Kauss and Hutchinson, 1975; Soto et al., 1975a; Parsons et al., 1976; Prouse et al., 1976; Winters et al., 1976). Low boiling point, soluble aromatics are thought to be mainly responsible for the high initial toxicity of crude oils. For example, Kauss et al. (1973) found marked stimulation of Chlorella growth in some oil extracts after the loss of toxic compounds through volatilization. These authors suggest that such stimulation results from an ability of some algae to use hydrocarbons in oils as metabolites or from by-products of bacterial metabolism (CO_2, NH_3). However, Vandermeulen and Ahern (1976) speculate that stimulation may instead be the result of carcinogenic stimulatory activity.

Field bioassays with distillates showed slight growth stimulation. The failure of distillates to inhibit phytoplankton growth in Clearwater Lake may be explained partly by the fact that the field enclosures were routinely bubbled with air to ensure mixing prior to sampling. However, UV absorbance measurements before and after bubbling indicated that loss of volatiles as a result of this treatment was slight. Increased bacterial populations in distillate enclosures may also have contributed to reduced distillate toxicity in the field. Similar increases of algae and bacteria in response to crude oil have been reported for freshwater ponds (Schindler et al., 1975). The detailed interpretation of the ATP analyses in these experiments is complicated and not fully understood. Increases in ATP in the second bioassay probably reflect increases in bacteria or rotifer growth (Figure 32), while declines in the third bioassay appear to be related to zooplankton mortality (Figure 34).

In the vicinity of coal storage piles, the effects of volatile organics will depend on both temperature and the opportunity for evaporative loss, as both of these variables will affect the rate of volatilization of toxic compounds (Vandermeulen and Ahern, 1976). As in the case of oil pollution (Prouse et al., 1976), the toxic effects of hydrocarbons from coal are likely to be short-lived unless inputs are maintained; and the interpretation of bioassay results is complicated by changing composition and concentrations of hydrocarbons with time, so that it is "impossible to ascribe a measured effect to a specific hydrocarbon or concentration."

Further study is necessary to determine the extent of the problem of algal bioaccumulation of organics from coal. Our work is in agreement with that of Thompson and Eglinton (1976) with crude oil in suggesting that diatoms accumulate aliphatics from fossil fuels. However, algal uptake of aromatics upon exposure to specific aromatic compounds has also been demonstrated (Payer and Soeder, 1975; Soto et al., 1975b; Walsh et al., 1977), and additional work might demonstrate the accumulation of aromatics from coal volatilization products.

Several problems arise in using the results reported here to predict the environmental effects of coal storage facilities. Our results are reported in terms of volume percent additions of standard leachates and distillates. Yet these "standard" test materials exhibited considerable variation in physical and chemical characteristics, depending on variation in coal samples from which they were prepared. Coal samples obtained from different western mines differed in their products of leaching and volatilization. In addition, the resemblance of our leachates and distillates to materials actually entering the aquatic environment from coal piles is unknown. Considering the very high coal/water ratios existing in coal storage piles, it seems likely that concentrations of dissolved and particulate materials in our laboratory-prepared leachates and distillates were unrealistically low. There is a need for site-specific studies of coal storage facilities which are designed to assess both the quality and quantity of runoff.

REFERENCES

1. American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14th ed. APHA, New York. 1193 pp.

2. American Society of Testing and Materials. 1976. Annual book of ASTM standards. Part 31, Sec. D, 5-12. ASTM, Philadelphia. pp. 274-5.

3. Atkinson, L.P., W.M. Dunstan, and J.G. Natoli. 1977. The analysis and control of volatile hydrocarbon concentrations (e.g., benzene) during oil bioassays. Water, Air, and Soil Pollution 8: 235-242.

4. Carlson, R.M., A.R. Oyler, E.H. Gerhart, R. Caple, K.J. Welch, H.L. Kopperman, D. Bodenner, and D. Swanson. 1978. Environmental implications of polynuclear aromatic hydrocarbons liberated from northern Great Plains coal. Final report. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minn. 169 pp.

5. Chau, Y.K. and K. Lum-Shu-Chan. 1974. Determination of labile and strongly bound metals in lake water. Water Research 8: 383-388.

6. Coward, N.A., J.W. Horton, R.G. Koch, and R.D. Morden. 1978. Static coal storage--biological and chemical effects on the aquatic environment. Final report. Environmental Protection Agency, Duluth, Minn. 169. pp.

7. Dunstan, W.M., L.P. Atkinson, and J. Natoli. 1975. Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. Marine Biology 31: 305-310.

8. Ellis, B.E. 1977. Degradation of phenolic compounds by fresh-water algae. Plant Science Letters 8: 213-216.

9. Environmental Protection Agency. 1971. Algal assay procedure bottle test. National Eutrophication Research Program, U.S. EPA. 82 pp.

10. Gerhart, D.Z., S.M. Anderson, and J. Richter. 1977. Toxicity bioassays with periphyton communities: design of experimental streams. Water Research 11: 567-570.

11. Gerhart, D.Z. and T.E. Davis. 1978. Effect of heavy metals and sulfur dioxide on phytoplankton. Final report. Minnesota Regional Copper-Nickel Study, Minneapolis. 44 pp.

12. Gordon, D.C., Jr. and N.J. Prouse. 1973. The effects of three oils on marine phytoplankton photosynthesis. Marine Biology 22: 329-333.

13. Holm-Hansen, O. and C.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnology and Oceanography 11: 510-519.

14. Ishio, S., K. Kawabe, and T. Tomiyama. 1972. Algal cancer and its causes. I. Carcinogenic potencies of water and suspended solids discharged into the River Ohmuta. Bulletin of the Japanese Society of Scientific Fisheries 38: 17-24.

15. Ishio, S., H. Nakagawa, and T. Tomiyama. 1972. Algal cancer and its causes. II. Separation of carcinogenic compounds from sea bottom mud polluted by wastes of the coal chemical industry. Bulletin of the Japanese Society of Scientific Fisheries 38: 571-576.

16. Kauss, P., T.C. Hutchinson, C. Soto, J. Hellebust, and M. Griffiths. 1973. The toxicity of crude oil and its components to freshwater algae. In: Proceedings of Joint Conference on Prevention and Control of Oil Spills, March 13-15, Washington, D.C. pp. 703-714.

17. Kauss, P.B. and T.C. Hutchinson. 1975. Studies on the susceptibility of Ankistrodesmus species to crude oil components. Verh. Internat. Verein. Limnol. 19: 2155-2164.

18. Mattson, J.S., C.A. Smith, T.T. Jones, S.M. Gerchakov, and B.D. Epstein. 1974. Continuous monitoring of dissolved organic matter by UV-visible photometry. Limnology and Oceanography 19: 530-535.

19. Mrkva, M. 1969. Investigation of organic pollution of surface waters by ultraviolet spectrophotometry. Journal of the Water Pollution Control Federation 41: 1923-1931.

20. Niemi, A. 1972. Effects of toxicants on brackish-water phytoplankton assimilation. Commentat. Biol. 55: 1-19.

21. Parsons, T.R., W.K. Li, and R. Waters. 1976. Some preliminary observations on the enhancement of phytoplankton growth by low levels of mineral hydrocarbons. Hydrobiologia 51: 85-89.

22. Payer, H.D. and C.J. Soeder. 1975. Accumulation of polycyclic aromatic hydrocarbons in cultivated microalgae. Naturwissenschaften 62: 536-537.

23. Prouse, N.J., D.C. Gordon Jr., and P.D. Keizer. 1976. Effects of low concentrations of oil accommodated in sea water on the growth of unialgal marine phytoplankton cultures. Journal of the Fisheries Research Board of Canada 33: 810-818.

24. Pulich, W.M., Jr., K. Winters, and C. Van Baalen. 1974. The effects of a no. 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. Marine Biology 28: 87-94.

25. Rudd, J.W.M. and R.D. Hamilton. 1973. Measurement of adenosine triphosphate (ATP) in two Precambrian Shield lakes of Northwestern Ontario. Journal of the Fisheries Research Board of Canada 30: 1537-1546.

26. Samuelsson, G., G. Oquist, and P. Halldal. 1978. The variable chlorophyll a fluorescence as a measure of photoshythetic capacity in algae. Mitt. Internat. Verein. Limnol. 21: in press.

27. Schindler, D.B., B.F. Scott, and D.B. Carlisle. 1975. Effect of crude oil on populations of bacteria and algae in artificial ponds subject to winter weather and ice formation. Verh. Internat. Verein. Limnol. 19: 2138-2144.

28. Slovacek, R.E. and P.J. Hannan. 1977. In vivo fluorescence determinations of phytoplankton chlorophyll a. Limnology and Oceanography 22: 919-924.

29. Soto, C., J.A. Hellebust, T.C. Hutchinson, and T. Sawa. 1975. Effect of naphthalene and aqueous crude oil extracts on the green flagellate Chlamydomonas angulosa. I. Growth. Canadian Journal of Botany 53: 109-117.

30. Soto, C., J.A. Hellebust, and T.C. Hutchinson. 1975. Effect of naphthalene and aqueous crude oil extracts on the green flagellate Chlamydomonas angulosa. II. Photosynthesis and the uptake and release of naphthalene. Canadian Journal of Botany 53: 118-126.

31. Stadelmann, P. 1974. Biomass estimation by measurement of adenosine triphosphate. In: R.A. Vollenweider (ed.), A manual on methods for measuring primary production in aquatic environments. Blackwell, London. pp. 26-30.

32. Strickland, J.D.H. and T.R. Parsons. 1968. A practical handbook of seawater analysis. Bulletin 167, Fisheries Research Board of Canada, Ottawa. 311 pp.

33. Thompson, S. and G. Eglinton. 1976. The presence of pollutant hydrocarbons in estuarine epipelic diatom populations. Estuarine and Coastal Marine Science 4: 417-425.

34. Vandermeulen, J.H. and T.P. Ahern. 1976. Effect of petroleum hydrocarbons on algal physiology: review and progress report. In: A.P.M. Lockwood (ed.), Effects of pollutants and aquatic organisms. Cambridge University Press. pp. 107-125.

35. Vollenweider, R.A. (ed.) 1974. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12, 2nd edition. Blackwell, London. 225 pp.

36. Walsh, G.E., K.A. Ainsworth, and L. Faas. 1977. Effects and uptake of chlorinated naphthalenes in marine unicellular algae. Bulletin of Environmental Contamination and Toxicology 18: 297-302.

37. Winters, K., R. O'Donnell, J.C. Batterton, and C. Van Baalen. 1976. Water-soluble components of four fuel oils: chemical characterization and effects on growth of microalgae. Marine Biology 36: 269-276.

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